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CONTRIBUTOS PARA O ESTUDO DA CLOROSE FÉRRICA

Teresa Maria Rego Saavedra

Relatório de Atividade Profissional apresentado para a obtenção do grau de Mestre pelos licenciados Pré-Bolonha, enquadrado no Despacho RT.033/2011.

MESTRADO EM HORTOFRUTICULTURA

Relatório efetuado sob a orientação da Professora Doutora Maribela Pestana Correia

2013

CONTRIBUTOS PARA O ESTUDO DA CLOROSE FÉRRICA

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Teresa Maria Rego Saavedra

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Embora este relatório de atividade profissional, pela sua finalidade académica, seja um trabalho individual, há contributos de natureza diversa que não podem e nem devem deixar de ser realçados.

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RESUMO

O Relatório de Atividade Profissional agora apresentado reflete o meu percurso profissional desde 2007, no qual exerci sempre funções relacionadas com a minha área de formação, Hortofruticultura e noutras áreas complementares face às técnicas laboratoriais que aprendi.

Neste âmbito, o tema de trabalho escolhido -C*ontributos para o estudo da clorose férrica*- destaca a importância do ferro (Fe), que apesar de ser necessário em pequenas quantidades pelas plantas, a incidência de clorose férrica (deficiência de Fe) é comum em muitas espécies agrícolas sendo necessário recorrer à aplicação ao solo de quelatos de Fe sintéticos. Neste capítulo, faço um breve enquadramento teórico sobre o que se investiga nesta área e toda a problemática da clorose férrica, indico os ensaios e respetivas metodologias em que estive envolvida e apresento, de forma resumida, os resultados obtidos em diversos ensaios de caraterização deste desequilíbrio nutricional e de estudo de novas alternativas para a correção da clorose férrica. Em todos os ensaios, conduzidos em sistema hidropónico em que participei, os sintomas foram induzidos pela ausência do Fe na solução nutritiva e os resultados comparados com um tratamento controlo com Fe. O grau de clorose e a recuperação dos sintomas foram estimados através dos valores de SPAD. A atividade da quelato de Fe(III)-redutase (QF-R), enzima responsável pela redução do Fe nas raízes, foi determinada nos ápices radiculares pela quantificação colorimétrica do complexo Fe (II) -BPDS.

Executei diversos ensaios onde determinei a qualidade, interna e externa, dos frutos. Ainda colaborei na determinação da composição mineral de diverso material vegetal. Participei na validação de um extrato vegetal preparado a partir de aparas de relva (conforme descrição detalhada na patente PT/103584-2009 da UALG e na patente internacional PCT/PT2007/000041-2008; em copropriedade entre a UALG e a empresa ADP-Adubos de Portugal S.A.) e que foi eficaz na recuperação dos sintomas de clorose férrica e que poderá vir a ser alternativa ao uso dos quelatos férricos sintéticos.

Os trabalhos que desenvolvi no âmbito da minha atividade profissional abriram novas perspetivas de estudo da clorose férrica e introduziram melhorias nas técnicas de fertilização de fruteiras.

Palavras – chave: hidroponia, clorose férrica, morangueiro, SPAD, qualidade da produção.

ABSTRACT

The Professional Activity Report now presented reflects my professional career since 2007, in which I always exercised functions related to my area of training, horticulture and other complementary areas related to the laboratory techniques I learned.

In this context, the chosen theme -*Contributions to the iron chlorosis study* - highlights the importance of iron (Fe), although it is required in small amounts by plants, the incidence of iron chlorosis (Fe deficiency) is very common in a number of crops and requires massive soil application of Fe-chelates to correct it. In this chapter, I make a brief theoretical framework wherein this area investigates and the whole issue of iron chlorosis, I indicate the respective experiments and methodologies that I have been involved, and summarize the results obtained in several experiments of this nutritional imbalance characterization and study of new alternatives to correct iron chlorosis. In all experiments, conducted in hydroponic systems in which I participated, the symptoms were induced by withdrawing Fe from the solution and the results were compared to a control treatment grown with Fe. The degree of chlorosis and recovery symptoms was estimated using SPAD values. The activity of iron chelate reductase, the enzyme responsible for Fe reduction in roots, was determined in root apices by colorimetric quantification of the BPDS complex.

I executed several experiments which determined the internal and external quality of the fruit, and I also collaborated in determining the mineral composition of different plant material. I participated in the validation of a plant extract obtained from fresh grass clippings (national patent PT/103584-2009 of UALG, and international patent PCT/PT2007/000041-2008, UALG and ADP- ADP-Adubos de Portugal S.A.) which was effective in the recovery of iron chlorosis symptoms that could be an alternative to the use of synthetic ferric chelates.

The tasks that I developed within my professional activity have opened new perspectives for the study of iron chlorosis and introduced improvements in the techniques of fertilization of fruit trees.

Keywords: hydroponic, iron chlorosis, strawberry, SPAD, quality production.

ÍNDICE

I. INTRODUÇÃO

A componente letiva do Mestrado de Hortofruticultura a que me candidatei foi creditada pela Comissão do Mestrado e validada pelo Conselho Cientifico da Faculdade de Ciências e Tecnologia (FCT) da Universidade do Algarve (UALG). A Comissão do Mestrado propôs que eu apresentasse um Relatório de Atividade Profissional, complementado pelo meu *Curriculum* profissional por forma a obter o grau de Mestre em Hortofruticultura, após discussão pública.

Assim, no presente relatório apresento e desenvolvo a formação e as competências profissionais que obtive após finalizar a licenciatura em 2007. Até à data tenho vindo a desenvolver a minha atividade profissional integrada como bolseira de investigação ou em regime de prestação de serviços, nos projetos de investigação e de experimentação em curso no Laboratório de Nutrição Vegetal da FCT na UALG. Durante este período contactei com uma diversidade de assuntos e de situações, que possibilitaram o meu enriquecimento profissional que me proponho a discutir de forma detalhada no decurso deste relatório.

Neste âmbito escolhi o tema "*Contributos para o estudo da clorose férrica*" por considerar que grande parte da minha atividade foi desenvolvida nesta área, destacando-se a importância do Fe como micronutriente, essencial para as plantas. Assim, inicio o Relatório com uma breve reflexão sobre a importância do papel do Fe na planta, descrevendo de seguida algumas das metodologias que aprendi e executo no âmbito da minha participação nos diversos ensaios, apresentando alguns resultados com interesse no sector hortofrutícola. No último capítulo descrevo a minha experiencia profissional, destacando os artigos de que sou coautora, os quais anexo no final deste relatório.

II. CONTRIBUTOS PARA O ESTUDO DA CLOROSE FÉRRICA

1. ENQUADRAMENTO TEÓRICO: A DINÂMICA DO FERRO NA PLANTA

Considera-se indispensável estudar, de forma dinâmica, o papel dos elementos nutritivos na planta salientando a sua participação nas diversas reações bioquímicas envolvidas no crescimento e na produção. De entre estes elementos minerais, o Fe é um micronutriente essencial para as plantas superiores, na medida em que quando não se encontra disponível em quantidades suficientes é suscetível de limitar severamente as produções agrícolas (Álvarez-Fernández et al., 2003; 2006; Pestana et al., 2003).

O Fe é um metal de transição que pode mudar facilmente o seu estado de oxidação recebendo ou doando eletrões ($Fe^{3+} + e^{-} \leftrightarrow Fe^{2+}$), participando deste modo em vários processos fisiológicos tais como, a fotossíntese, a respiração, a redução do ião nitrato e a fixação biológica do azoto (Varennes, 2003). O processo de absorção do Fe nas dicotiledóneas inicia-se pela redução na membrana plasmática do Fe(III) existentes na solução do solo (Marschner e Römheld, 1986), através da ação da quelato de Fe(III) -redutase (QF-R). Esta enzima tem a sua atividade máxima em meio ácido (pH próximo de 5.0), sendo inibida se a reação for alcalina (Abadía et al., 2011). O Fe é transportado, via xilema, para a parte aérea complexado pelo ácido cítrico (Pestana et al., 2003). Já na parte aérea, o Fe (III) é de novo reduzido por outra QF-R existente no plasmalema das folhas, cuja atividade é máxima para valores de pH entre 5.5 e 6.0 (González-Vallejo et al., 2000).

Quando a absorção ou os processos fisiológicos em que participa se encontram reduzidos ou inativos, as plantas apresentam sintomas desta deficiência nutritiva, que devido à baixa mobilidade do Fe surgem primeiro nas folhas mais jovens, caracterizando-se pelo aparecimento de um fino reticulado no qual apenas as nervuras permanecem verdes (Figura 2.1 A), podendo em estados de deficiência grave, as folhas ficarem brancas, totalmente desprovidas de clorofila (Figura 2.1 B). O termo clorose férrica é aplicado à manifestação dos sintomas típicos de deficiência de Fe (Pestana et al*.,* 2004a).

As folhas cloróticas não produzem fotoassimilados suficientes para o crescimento e desenvolvimento adequado de raízes, pecíolos, ramos, folhas e frutos (Álvarez-Fernández et al*.*, 2006; Abadía et al., 2011) pelo que, a qualidade do fruto e a produção agrícola, pode ser bastante afetada (Pestana et al*.,* 2003; 2004a).

O conhecimento dos fatores indutores e dos efeitos da clorose férrica no metabolismo vegetal permite estabelecer métodos de diagnóstico e de correção desta deficiência nutritiva, minimizando as implicações ambientais e económicas inerentes a esta deficiência (Pestana et al. 2004b; 2005; 2011; 2012).

Figura 2.1: Imagem ilustrativa das folhas de morangueiro com sintomas típicos **(A)** e sintomas acentuados de clorose férrica **(B)**, comparativamente a folhas sem sintomas **(C)**.

O ião bicarbonato (HCO_3^-) é um dos fatores de indução de clorose férrica, principalmente em solos calcários (Varennes, 2003) e resulta da dissociação do $CaCO_3$ que ao aumentar o pH, afeta a atividade radicular da QF-R. A inibição da aquisição de Fe pela presença do ião bicarbonato pode ser agravada se a este fator, se associarem outros desfavoráveis à absorção do Fe como a compactação do solo, baixas temperaturas, presença do ião nitrato e disponibilidade de outros nutrientes. A compactação associada a baixas temperaturas do solo mantêm o teor de humidade excessiva (alagamento; presença de CO_2) por períodos de tempo mais longos, levando ao aumento da quantidade de HCO_3^- na solução do solo (Marschner, 2011).

Os diferentes mecanismos de resposta que contribuem para um melhor estado nutricional em Fe, logo para uma maior tolerância à clorose férrica, atuam através da mobilização do Fe na rizosfera e do aumento da taxa de absorção e de translocação deste elemento na planta (Abadía et al., 2011). Estes são ativados sempre que a concentração de Fe nos tecidos vegetais decresce abaixo do nível crítico, sendo desativados quando o nível de Fe necessário para a planta é alcançado, de forma a evitar a absorção deste elemento em excesso pela planta (Marschner, 2011).

O material vegetal é determinante no nível de tolerância das culturas, havendo espécies bem adaptadas a estas condições e que dificilmente desenvolvem sintomas de clorose férrica (Correia et al., 2003; Pestana et al., 2011a; 2012b). As plantas eficientes desenvolvem mecanismos específicos de resposta, que incluem alterações fisiológicas e morfológicas, tanto ao nível da parte aérea como da parte radicular, e que podem ser divididos em duas estratégias: Estratégia I, encontrada em dicotiledóneas e em algumas monocotiledóneas e Estratégia II, característica das gramíneas que libertam fitosideróferos para a rizosfera e possuem simultaneamente um sistema de absorção do Fe com elevada especificidade para os fitosideróferos férricos formados (Marschner e Römheld, 1986).

As plantas agrupadas na Estratégia I respondem à carência de Fe através de alterações fisiológicas e morfológicas ao nível radicular. Observa-se o incremento da atividade da QF-R, das

ATPases e da libertação de substâncias redutoras e quelatantes do Fe na rizosfera (Abadía et al., 2011). As alterações morfológicas incluem um incremento da formação de raízes laterais e de pelos radiculares (Figura 2.2A), com o consequente aumento da superfície de redução e absorção do Fe (Marschner e Römheld, 1986; Pestana et al., 2012a; 2012b).

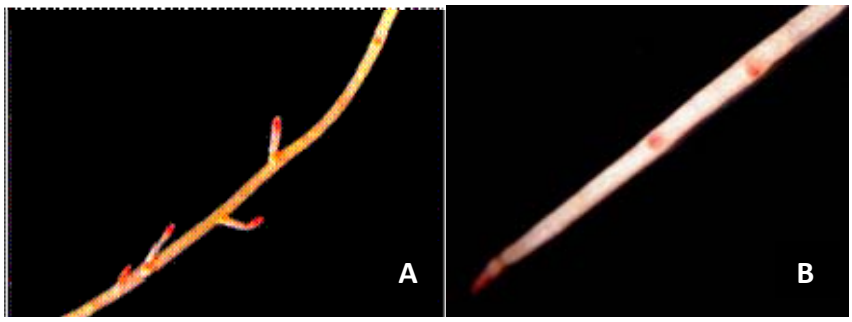


Figura 2.2: Imagem ilustrativa da alteração morfológica externa dos ápices radiculares de uma planta de morangueiro com sintomas de clorose férrica **(A),** comparativamente a uma planta controlo sem sintomas **(B).** Ampliação 6x. Fonte: (Pestana et al., 2012a).

Diversos autores (Álvarez-Fernández et al., 2003; 2006; Pestana et al., 2004b; 2005; Abadía et al., 2011) salientam a importância de um diagnóstico precoce, que antecipe o aparecimento dos sintomas e permita corrigir atempadamente os efeitos negativos da clorose férrica na qualidade do fruto. Como métodos de diagnóstico incluem-se as análises ao solo e análises foliares (Varennes, 2003).

No entanto, as análises ao solo podem ser limitantes quando aplicadas a fruteiras já que estas apresentam o radicular profundo, irregularmente distribuído, que dificulta a obtenção de uma amostra representativa da disponibilidade nutritiva, aspeto agravado nos solos calcários (Pestana et al., 2003).

A análise foliar é o método de diagnóstico mais utilizado para identificação de clorose férrica pois integra os fatores que podem influenciar a disponibilidade do Fe no solo e na planta no momento da colheita (Pestana et al., 2003). No entanto, o uso da concentração foliar de Fe quando a clorose férrica é induzida em solos calcários, pode estar limitado pelo como o "paradoxo do ferro" (Römheld, 2000), já que as folhas cloróticas podem muitas vezes apresentar, especialmente em campo, concentrações de Fe superiores às das folhas verdes o que pode dever-se à imobilização do Fe a nível das nervuras.

A análise floral foi testada em diversas fruteiras (pessegueiro, macieira, pereira, laranjeira, kiwi, entre outras) estando, no entanto, a sua aplicabilidade limitada. Em campo, o diagnóstico da clorose férrica deve resultar da integração dos diferentes métodos.

Atualmente, a correção da clorose férrica em fruteiras faz-se sobretudo recorrendo a aplicações ao solo de quelatos férricos sintéticos, que têm de se repetir anualmente para o mesmo pomar, pois o Fe aplicado num ano não previne o aparecimento dos sintomas de carência no ano seguinte (Pestana et al., 2003; 2005; 2011b; 2012a). Para além dos custos associados a esta prática, os impactos ambientais da aplicação de quelatos ao solo parece ter um impacto negativo pois promove a absorção de outros metais como o manganês (Mn), o cobre (Cu) e o níquel (Ni). Como alternativa ao uso de quelatos, têm sido estudados métodos de correção da clorose férrica mais sustentáveis, tanto em termos económicos como ambientais (Pestana et al., 2003; 2011b; 2012b).

As pulverizações foliares são uma alternativa com inferior impacto ambiental, para o controlo da clorose férrica. O sucesso dos tratamentos foliares com Fe depende da capacidade destes em penetrarem a cutícula, atravessarem a zona do apoplasto, o plasmalema e atingirem o citoplasma das células foliares (Rombolà et al., 2000; Pestana et al., 2012b). De um modo geral, os tratamentos foliares são apenas eficazes quando usados em plantas com sintomas ligeiros ou moderados de clorose férrica e os efeitos são de curta duração sendo necessário repetir várias vezes os tratamentos para manter o reverdecimento foliar, o que encarece esta opção (Rombolà et al., 2000).

Em conclusão, a clorose férrica é um desequilíbrio nutricional que afeta imensas fruteiras (citrinos, kiwi, pereira, pessegueiro, macieira, cerejeira, entre outras), com especial ênfase na bacia Mediterrânica, afetando a qualidade do fruto, sendo necessário recorrer a práticas culturais adaptadas a esses pomares. O aparecimento da clorose férrica obriga a um ajuste das fertilizações para minimizar o impacto desta deficiência nutricional na produtividade e qualidade ambiental. A investigação e experimentação em morangueiro podem contribuir para o conhecimento nesta área.

2. METODOLOGIAS DESENVOLVIDAS PELA CANDIDATA

Neste item apresento algumas das técnicas que aprendi e executei após a finalização da licenciatura.

2.1 HIDROPONIA

No decurso da minha atividade participei em vários ensaios estabelecidos em hidroponia na UALG, essenciais ao estudo da deficiência de Fe e mais tarde de recuperação dos sintomas. Estes ensaios decorreram o âmbito dos projetos PTDC/AGR-ALI/66065/2006, PTDC/AGR-AAM/100115/2008 e de outras colaborações científicas, tendo eu tido a possibilidade de aprender técnicas das culturas sem solo, um importante modo de produção em horticultura. Para a instalação dos ensaios em hidroponia aprendi diversas técnicas em laboratório como a preparação de soluções "stock", diluições de soluções, e assumi a gestão dos recursos (reagentes, material de vidro e equipamento) necessários a estes procedimentos.

Os ensaios decorreram maioritariamente numa estufa de vidro localizada no 'Horto' da Universidade do Algarve (UALG) no *Campus* de Gambelas. As plantas foram colocadas em solução nutritiva, em caixas de plásticos opacas de 20 L, ou em frascos de vidro de 1 L. A solução nutritiva utilizada nestes ensaios foi a de Hoagland, realizada com água desmineralizada e com as seguintes concentrações (mM): 5.0 Ca $(NO_3)_2.4H_2O$, 5.0 KNO_3, 1.0 KH_2PO_4, 2.0 $MgSO_4.7H_2O$, e (µM) 46.0 H_3BO_3, 0.8 $ZnSO_4.7H_2O$, 0,4 $CuSO_4.5H_2O$, 9.0 $MnCl_2.4H_2O$ e 0.02 MoO_3. O Fe foi adicionado em diversas formas e concentrações de acordo com as diferentes culturas e objetivos estabelecidos a que corresponderam diferentes modalidades testadas, resumidas na Tabela 2.1. Após a preparação da solução nutritiva acertaram-se os valores de pH para próximo de 6,0 utilizando NaOH (1N) e/ou HNO_3 (10%) e mediu-se a condutividade elétrica (CE). Neste contexto também trabalhei com equipamentos e outras técnicas nomeadamente, potenciometria e condutimetria. Assegurei a manutenção das soluções nutritivas ao longo dos ensaios, adicionando água desmineralizada, que só substituí quando os valores de pH e de condutividade elétrica (CE) baixaram. O arejamento destas soluções nutritivas foi garantido por um compressor ligado a um sistema de tubagens, regulado por torneiras de forma a garantir um fluxo de ar adequado a nível radicular.

Tabela 2.1: Descrição dos tratamentos de indução de sintomas de clorose férrica e posterior recuperação realizados em cultivares de morangueiro, com diferentes modos de aplicação e fontes de Fe.

Projeto PTDC/AGR-ALI/66065/2006						
Morangueiro cultivar	**Indução sintomas (dias)**	**Tratamentos [µM] Fe**	**Tratamentos de recuperação**	**Modo de aplicação**	**Concentração $FeSO_4$ / Fe-EDDHA**	**Recuperação (dias)**
Selva	53	- (Fe): 0 +(Fe):10	+ $FeSO_4$	À solução Pulverização foliar (3X)	0,75 mM 1,8 mM	17
Diamante	33	- (Fe): 0 +(Fe):10	+ Fe-EDDHA + $FeSO_4$	À solução Aplicação foliar localizada	10 µM 2 mM	15

Efetuei ainda o transplante dos morangueiros para as caixas (Figura 2.3) ou frascos com solução nutritiva, após lavagem e desinfeção das raízes por imersão numa calda com fosetil de alumínio, onde permanecem durante algumas horas. O controlo das pragas e doenças foi efetuado por mim e só, quando estritamente necessário, é que recorri a luta química, tendo o cuidado de usar apenas substâncias homologadas para Produção Integrada.

No decorrer destes ensaios instalei as diferentes modalidades definidas de acordo com o desenho experimental estabelecido em reunião de grupo (Laboratório de Nutrição Vegetal).

Determinei semanalmente vários parâmetros tais como, o número de folhas, o número de flores, o número de estolhos, o número de frutos e o teor de clorofila total das folhas, bem como a atividade da enzima QF-R. Efetuei todas as práticas culturais necessárias ao desenvolvimento da cultura incluindo as colheitas semanais e análise nutricional das plantas e frutos.

Figura 2.3: Aspeto geral dos ensaios com morangueiros em caixas de 20L. **A:** Morangueiros em solução nutritiva completa; **B:** Morangueiros com clorose férrica.

Instalei ainda ensaios em hidroponia com fruteiras lenhosas (Figura 2.4) o que envolveu outras metodologias e possibilitou o estudo das respostas fisiológicas a nível radicular. Como

exemplo, são apresentados ensaios com alfarrobeiras e porta-enxertos de citrinos, desenvolvidos no âmbito do projeto PTDC/AGR-AAM/100115/2008 (Tabela 2.2).

Tabela 2.2: Ensaios de hidroponia com diferentes concentrações de Fe na solução nutritiva.

Projeto PTDC/AGR-AAM/100115/2008			
Espécie	**Dias de ensaio**	**Concentrações de Fe [µM] Fe (Fe-EDDHA) (Tratamentos)**	**Parâmetros avaliados**
Alfarrobeira	72	- (Fe): 0 (Fe): 1 +(Fe):10	• SPAD • Calibração da clorofila total • Nº Folhas • Altura • Atividade da QF-R • Biomassas • pH e CE das soluções
***Poncirus trifoliata* L.**	64	- (Fe): 0 (Fe): 1 +(Fe):40	
Citrangeira *Troyer* **Citrumelo *Swingle***	25	- (Fe): 0 +(Fe):10	

Nos ensaios com alfarrobeiras usei plantas provenientes de viveiro, mas também plantas provenientes de sementeiras, realizadas em substrato inerte, nomeadamente em vermiculite. Devido à dureza do tegumento procedi à escarificação destas sementes com água quente.

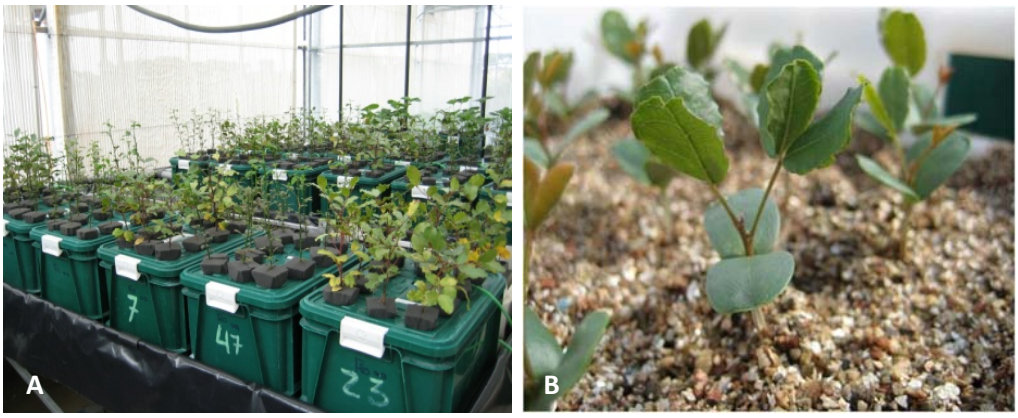


Figura 2.4: A: Aspeto geral do ensaio com alfarrobeiras e Poncirus *trifoliata* L.; **B:** Alfarrobeiras germinadas em vermiculite para posterior utilização nos ensaios.

2.2 ENSAIOS EM VASO

No âmbito do Acordo Específico de Licenciamento Exclusivo de Tecnologia estabelecido entre a UALG e a ADP-Adubos de Portugal S.A. realizei um ensaio com plantas de tomate de indústria instaladas em vasos preenchidos com solo calcário, ao ar livre. O objetivo deste ensaio foi comprovar a eficácia agronómica de aplicações foliares de extratos de relva, selecionados com base na sua eficácia de correção da clorose férrica.

Participei na instalação deste ensaio no 'Horto' da UALG, ao ar livre, usando plantas de tomate de indústria, colocadas em vasos com solo calcário (+ de 12% de calcário ativo). Para preencher cada vaso foram necessários cerca de 4,6 L de solo que foi previamente seco e crivado e no qual foi adicionado vermiculite, na proporção de 1:4, para melhorar a estrutura do solo que apresentava mais de 20% de argila.

Instalei o sistema de rega localizada e mantive as plantas, executando as práticas culturais necessárias. Testei várias pulverizações foliares, sendo a modalidade pulverizada com água a testemunha ou controlo clorótico, e a modalidade tratada com quelato férrico, o controlo verde. O extrato a partir das aparas de relvas foi produzido de acordo com o descrito nas patentes (patente nacional-103584 da UALG e na patente internacional da UALG em copropriedade com a ADP-Adubos de Portugal SA (PCT/PT2007/000041). As restantes modalidades não estão descritas por serem confidenciais e propriedade da empresa (Tabela 2.3).

Tabela 2.3: Ensaio em vaso de plantas de tomateiro.

Acordo Específico de Licenciamento Exclusivo de Tecnologia entre UALG e ADP-Adubos de Portugal		
Ensaio em vaso com solo calcário		
Espécie	**Dias de ensaio**	**Tratamentos**
Tomate de indústria	65	**C**: Água **Q**: Quelato de ferro (Fe-EDDHA) **E**: Extrato de relva; **O:** outros tratamentos que são confidenciais da empresa.

Avaliei a clorose férrica através da utilização do aparelho de SPAD-502 (Minolta) com leituras efetuadas três vezes por semana, imediatamente antes de cada pulverização.

Nestas datas também registei parâmetros de crescimento vegetativo, tais como a altura das plantas e o número de folhas, flores e frutos. No início e no final do ensaio, fui ainda responsável pelos estudos da biomassa e de composição mineral das plantas, pela análise estatística dos resultados e pela elaboração da proposta de relatório enviada à empresa com as principais

conclusões, as quais não poderão ser divulgadas face ao acordo de confidencialidade existente entre a empresa e a UALG.

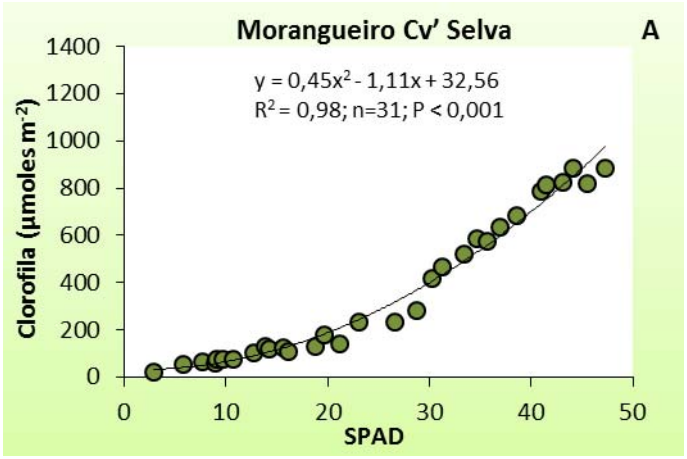
2.3 AVALIAÇÃO DOS SINTOMAS DE CLOROSE FÉRRICA

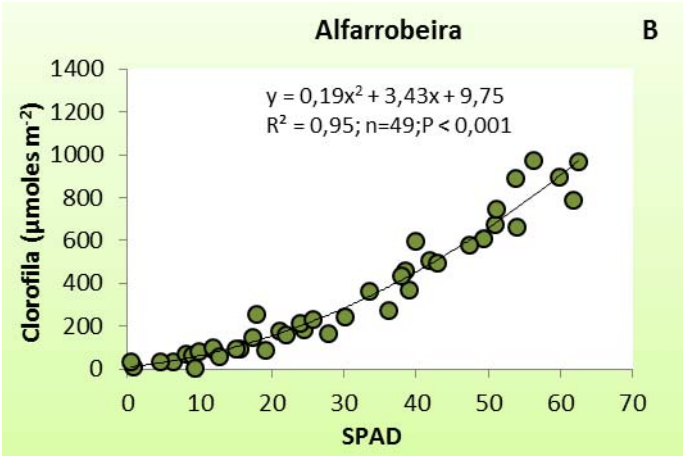
Neste período aprendi a utilizar o aparelho de SPAD-502 (Minolta Co., Osaka, Japão) que permite estimar o grau de clorose de plantas, avaliando a intensidade luminosa transmitida pela folha convertida em unidades de SPAD (Figura 2.5). Este aparelho pode ser utilizado como um método não destrutivo avaliando o grau de clorose pois os valores de SPAD são proporcionais à quantidade de clorofila total existente nas folhas.

Laboratorialmente, realizei diversas curvas de calibração, que permitem converter os valores de SPAD em concentração foliar de clorofila. A extração dos pigmentos foi realizada com acetona a 100% na presença de ascorbato de sódio em discos foliares com diferentes graus de clorose (Abadía e Abadía, 1993). Determinei a absorvância das amostras num espectrofotómetro UV Visível (UV-160 A, Shimadzu) a dois comprimentos de onda (661,6 e 644,8 nm). A conversão da absorvância em clorofila foi efetuada usando as equações referidas por Lichtenthaler (1987). Os valores de SPAD foram convertidos em µmoles de clorofila total por unidade de área (m^2) segundo a função que melhor se ajustou, metodologia referenciada por diversos autores (Abadía e Abadía 1993; Pestana et al., 2011).

Figura 2.5: Aparelho de SPAD.

Na Figura 2.6 apresento as curvas de calibração correspondentes a três espécies utilizadas nos ensaios: Morangueiro cv. 'Selva' (**A**), Alfarrobeira (**B**) e P*oncirus trifoliata* L. (**C)**.





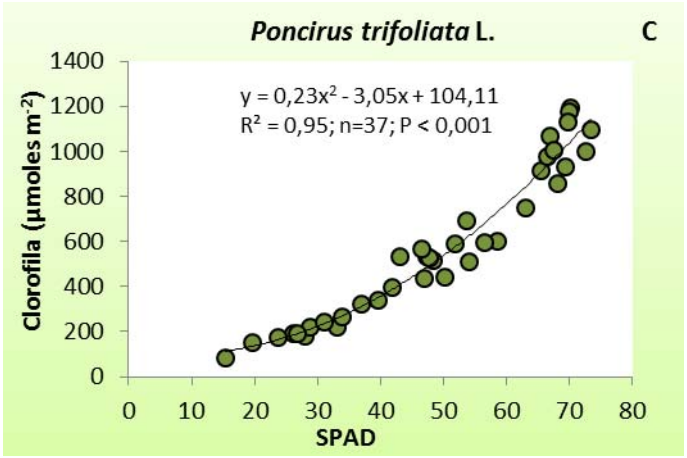


Figura 2.6: Relação entre o SPAD e a clorofila (µmoles m^{-2}) para morangueiro (Pestana et al., 2011) (**A**), alfarrobeira (**B**, dados não publicados) e *Poncirus trifoliata* (**C**, dados não publicados). Está indicado o coeficiente de correlação (R^2), o número de amostras (n) e o nível de significância (P).

2.4 ATIVIDADE DA QUELATO DE FE (III) – REDUTASE

Aprendi a determinação de atividades enzimáticas, nomeadamente da enzima QF-R em ápices radiculares de diversas espécies vegetais (Tabelas 2.1 e 2.2) usando o método modificado do proposto por Bienfait et al. (1983). Este método permite quantificar, por colorimetria o complexo Fe(II)-BPDS ('bathophenantrolinedisulfonate') resultante da redução do Fe adicionado na forma de quelato Fe(III)-EDTA. O procedimento está descrito em detalhe nos diversos artigos publicados (Correia et al., 2003; Pestana, 2000; Pestana et al., 2011a,b; 2012a,b) e utiliza ápices de raízes secundárias, cortadas com 2 cm de comprimento (Figuras 2.7A e 2.7B). Efetuei soluções de controlo (sem raízes) para eliminar a contaminação por fotoredução do Fe(III). Ajustei o tempo de reação no escuro às diferentes espécies estudadas; 1 hora, para o morangueiro e 2 horas para alfarrobeira e porta-enxertos de citrinos (*Poncirus trifoliata* L., Citrangeira "Troyer" e Citrumelo "Swingle")**.** Findo o tempo de reação procedi à leitura da absorvância a 535nm num espectrofotómetro UV-Visível (CADAS 100). No final tratei os resultados e apresento o gráfico da Figura 2.7A, como exemplo. Na

Figura 2.7B é possível observar o aspeto do Eppendorf após a formação do complexo com Fe reduzido de coloração rosa.

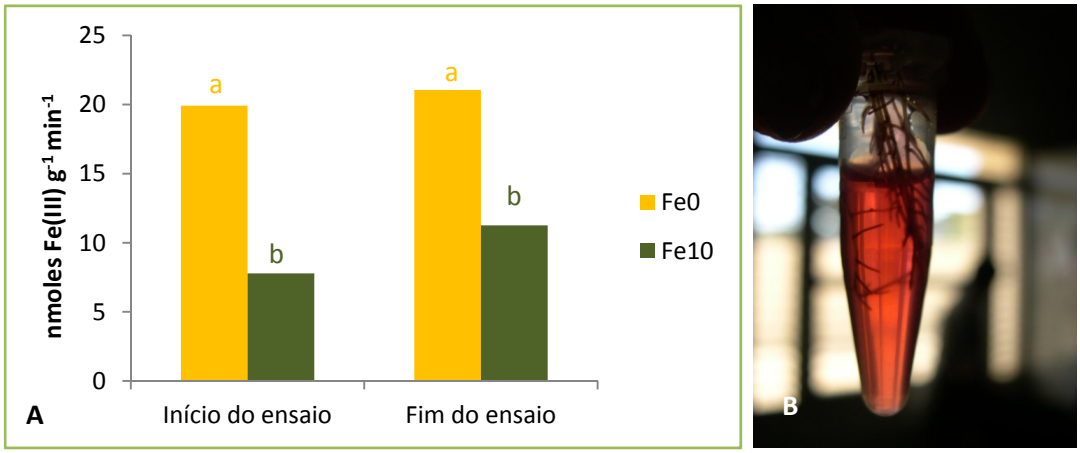


Figura 2.7: A. Exemplo da variação da atividade da enzima QF-R nos ápices radiculares de plantas de morangueiro no início e no fim do ensaio. Para cada data, pontos com a mesma letra não são significativamente diferentes (p ≥0,05). **B.** Fotografia de um Eppendorf com a solução que contém os ápices e o Fe reduzido (coloração rosa).

2.5 PARÂMETROS DE QUALIDADE DO FRUTO

No decurso do ensaios, e sempre que possível, avaliei a qualidade dos frutos colhidos, quer recorrendo a medições não invasivas (pela utilização do aparelho Vis/NIR) quer pelos métodos padrão. Em diversos ensaios de morangueiro determinei o peso fresco, o diâmetro longitudinal e transversal, a cor externa dos frutos, a firmeza, assim como o total de sólidos solúveis (º Brix), a acidez titulável e o pH do sumo dos frutos.

Determinei o peso fresco numa balança de precisão (0,001; BP 2100) e o calibre por medição direta do diâmetro transversal e do longitudinal do fruto com uma craveira digital (Mitutoyo), graduada em milímetros. Classifiquei a cor externa usando um colorímetro portátil (Minolta, CR-300), que regista a cor numa escala de Munsell, adaptada a frutos. Quantifiquei o total de sólidos solúveis, expresso em º Brix, através do índice de refração do sumo medido pela utilização de um refratómetro digital (Atago Palette, PR101) e determinei a firmeza dos frutos usando um penetrômetro manual (Bertuzzi) com percutor de 3,5 mm de diâmetro; deste modo, medi a resistência à compressão do êmbolo na zona equatorial do fruto. Efetuei a leitura do pH do sumo dos morangos (potenciómetro HI 9024C, Hanna Instruments) e calculei a acidez titulável pela neutralização dos ácidos do sumo com hidróxido de sódio (Figura 2.8).

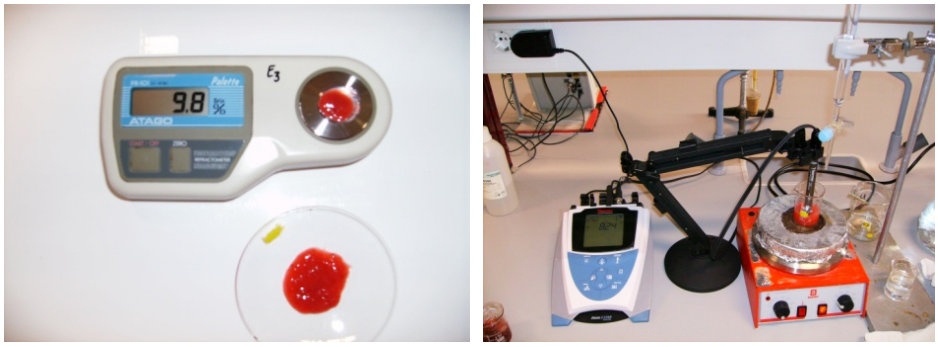


Figura 2.8: Determinação dos ºBrix através do refratómetro digital e do pH do sumo dos morangos.

A aprendizagem destas metodologias laboratoriais, entre outras contribuíram para o meu enriquecimento profissional e para a diversificação das minhas competências profissionais.

3. RESULTADOS OBTIDOS

Neste item apresento os resultados mais relevantes destacando as conclusões técnicas com relevância no sector Hortofrutícola.

3.1 MECANISMOS DE RESPOSTA

Quando os mecanismos de resposta a nível fisiológico não são suficientes para regularizarem a aquisição de níveis adequados de Fe pelas plantas, surgem os sintomas na parte aérea seguidos de alterações morfológicas ao nível da raiz, que correspondem a um aumento do número dos ápices laterais com a zona subapical dilatada observados ao longo destes ensaios (Pestana et al., 2012a). Tal como o morangueiro, também a alfarrobeira e os porta-enxertos de citrinos apresentaram suscetibilidade à ausência de Fe na solução nutritiva. No entanto, as plantas de alfarrobeira para o mesmo período de tempo de ensaio não apresentaram sintomas de deficiência de Fe e apresentavam valores de clorofila total semelhantes para todos os tratamentos. As alfarrobeiras cresceram sem sintomas durante mais tempo.

As diferenças observadas entre as espécies foram parcialmente explicadas pelo padrão de crescimento mais lento da alfarrobeira (Pestana et al., 2012b). Enquanto a atividade da enzima QF-R aumentou nas plantas de alfarrobeira que cresceram sem Fe (Fe0), embora não tenha apresentado quaisquer sintomas a nível foliar, no Poncirus *trifoliata* foi necessária uma pequena quantidade de Fe (Fe1) para que atividade da enzima QF-R incrementasse. Esta resposta enzimática diferencia

tolerâncias distintas e poderá vir a ser usado na seleção de porta-enxertos tolerantes à clorose férrica, em estádios muito juvenis encurtando os ensaios de campo, tradicionalmente usados no melhoramento vegetal.

3.2 A RECUPERAÇÃO DA DEFICIÊNCIA DE FERRO

Na Figura 2.9, estão apresentados os acréscimos de SPAD motivados pela adição de quelato férrico à solução nutritiva (Fe-EDDHA) com o intuito de corrigir a clorose férrica de morangueiros. A recuperação ou reverdecimento total das folhas novas (brancas) ocorreu passados 12 dias, após os quais os valores de SPAD nas folhas novas foram iguais aos das plantas que cresceram sempre com Fe.

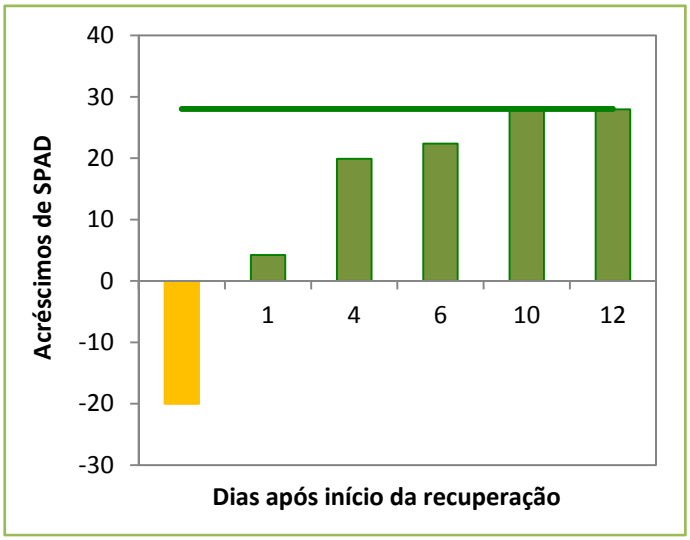


Figura 2.9: Acréscimos dos valores de SPAD nas folhas novas submetidos a diferentes modalidades ao longo do ensaio: com 10 µM de Fe (Fe10); sem Fe (Fe0); e recuperadas com 10 µM de Fe-EDDHA adicionada à solução.

Noutro ensaio, com morangueiros da cultivar 'Selva' em que testei a recuperação de morangueiros cloróticos através da adição de sulfato ferroso ($FeSO_4$) de dois modos: à solução nutritiva (+Fe solução) ou por três pulverizações foliares (+Fe folhas) foi possível distinguir as respostas obtidas. O $FeSO_4$ pode ser uma alternativa rápida de correcção da clorose férrica desde que adicionado à solução nutritiva, pois a recuperação dos sintomas foi evidente ao final de seis dias; já que nas plantas pulverizadas com $FeSO_4$ o reverdecimento foi apenas parcial.

Por sua vez, a atividade radicular da QF-R respondeu mais rapidamente ao Fe aplicado foliarmente, pois nestas plantas os valores decresceram e ficaram idênticos aos das plantas controlo

(que cresceram sempre com Fe). Por outro lado, a atividade da QF-R manteve-se alta mesmo depois da recuperação total dos sintomas nas folhas das plantas tratadas através da adição de Fe à solução, sugerindo uma possível oportunidade de incrementar as reservas deste elemento na planta.

Integrado na colaboração que a equipa do Laboratório de Nutrição Vegetal mantem com o CSIC-EEAD de Saragoça-Espanha no âmbito do Projeto Espanhol *Nuevos enfoques para el estúdio de la disponibilidad, movimiento y localización del Fe en la fertilización de árboles frutales* (AGL2009-09018), coordenado por Anunciación Abadía, tive ainda a oportunidade de estudar a mobilidade do Fe em folhas cloróticas de morangueiros (cv. Diamante e Primori), quando aplicado pontualmente em diferentes zonas da folha. Após o aparecimento dos sintomas, as folhas cloróticas foram parcialmente pinceladas com $FeSO_4$ do seguinte modo (Figura 2.10): só na parte basal (**Basal**) ou só na parte apical (**Apical**) do folíolo central, ou do lado esquerdo dos três folíolos longitudinalmente divididos pela nervura principal (**Longitudinal**). Usei os valores de SPAD para estimar o grau de clorose e de recuperação dos sintomas. No final do ensaio, independentemente da parte tratada com $FeSO_4$, o reverdecimento atingiu valores iguais ao controlo verde. A aplicação do fertilizante foliar foi particularmente efetiva na superfície das folhas e o reverdecimento acentuado. Quanto à zona não tratada, os valores de SPAD decresceram, tendo-se acentuado o grau de clorose. No final foi possível observar que, o movimento do Fe esteve confinado à zona tratada, que reverdeceu. Estes resultados salientam o padrão limitado do movimento do Fe na folha, permitindo delinear novas linhas de investigação na área da adubação foliar.

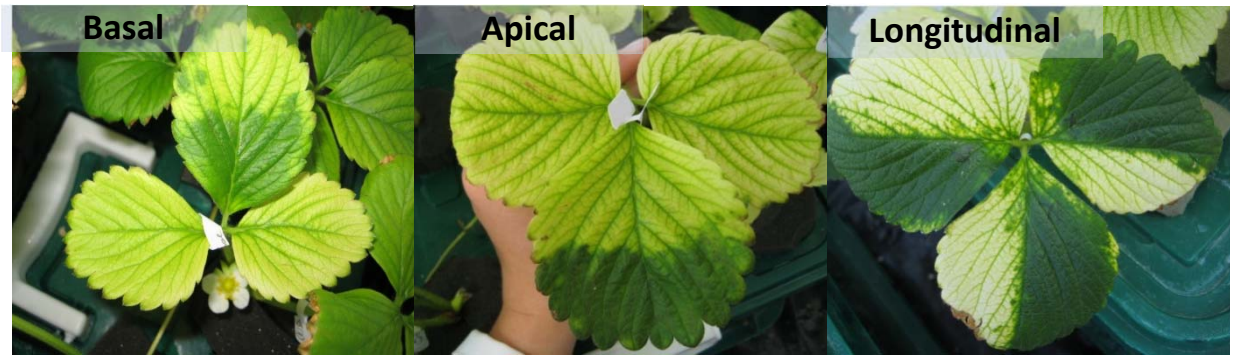


Figura 2.10: Imagens ilustrativas do reverdecimento das folhas de morangueiro após aplicação foliar localizada de $FeSO_4$.

Como resultado a destacar da minha participação no Acordo Específico de Licenciamento Exclusivo de Tecnologia entre a UALG e a ADP-Adubos de Portugal, posso referir que a aplicação foliar do extrato vegetal preparado a partir de aparas de relva pode ser uma possível alternativa aouso de quelatos férricos sintéticos na correção da clorose férrica. Na Figura 2.11 apresento uma

fotografia do ensaio em vaso, com solos calcários e usando plantas de tomate de indústria, conforme anteriormente descrito.

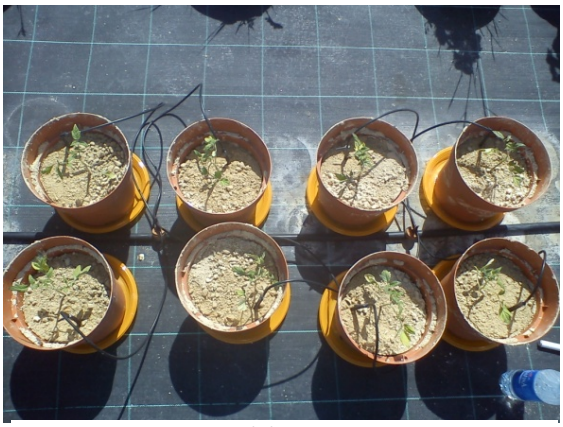


Figura 2.11: Aspeto geral dos tomateiros em vaso, ao ar livre.

O extrato proporcionou um incremento dos valores de SPAD que foram significativamente superiores aos do controlo, sendo possível otimizar a época de aplicação do Fe, minimizando as perdas e desenvolver novos métodos que permitam dinamizar as reservas nativas de Fe na planta.

3.3 EFEITO DA CLOROSE FÉRRICA NA QUALIDADE DO FRUTO

A clorose férrica origina um desequilíbrio nutricional na planta e na partição dos fotoassimilados afetando, consequentemente, a produção total, o calibre dos frutos, assim como a qualidade interna dos frutos.

Nos ensaios que acompanhei constatou-se que, no primeiro ciclo de produção, os morangueiros cloróticos produziram mais frutos maduros, e em alguns casos mesmo com mais peso total do que os frutos provenientes das plantas verdes (bem nutridas). No entanto, como os morangueiros permaneceram a crescer sem Fe, as plantas cloróticas no final do ensaio produziram menos e com pior qualidade, pois ocorreu o esgotamento das reservas endógenas. Estes resultados permitiram concluir que a deficiência em Fe, mesmo que temporariamente, afeta negativamente o ciclo vegetativo e, posteriormente o ciclo reprodutivo da cultura de morangueiro.

Figura 2.12: Frutos usados na determinação da qualidade. Detalhe de um morango maduro, à data de colheita.

Resumindo, a clorose férrica antecipou a entrada em floração e em produção e, embora tenha originado um maior número de frutos por planta no primeiro ciclo de produção, estes foram mais pequenos e de qualidade inferior pertencendo todos à categoria III (diâmetro inferior a 18mm).

Por outro lado, as plantas verdes produziram menos frutos. Estes resultados poderão ter a ver com a partição de biomassa e o investimento preferencial das plantas cloróticas no crescimento reprodutivo em detrimento do vegetativo.

3.4 OUTROS ENSAIOS

Fora do tema selecionado para este relatório participei ainda em diversos ensaios, realçando aqui apenas alguns dos principais resultados obtidos:

- ***Efeito do cálcio (Ca) na incidência do tipburn em morangueiro:***

O *tipburn* é geralmente considerado como uma desordem fisiológica causada pela deficiência de Ca que se traduz por uma necrose nas margens das folhas jovens (Saure, 1998).

Figura 2.13: Exemplos de *tipburn* em plantas de morangueiro.

Colaborei na instalação de ensaios em substrato orgânico (fibra de coco, com casca de pinheiro e turfa) e sistema de fertirrega (Figura 2.14); os tratamentos, com diferentes níveis de Ca foram adicionados por um sistema de rega localizada (Figura 2.14A) ou por pulverização foliar (Figura 2.14B). Como resultados destacam-se que, para o conjunto das três cultivares estudadas a percentagem de incidência do *tipburn* não esteve relacionada com a aplicação de Ca no substrato, tendo aumentado ao longo do tempo e tendo sido mais intensa no final do ensaio, coincidindo com o período de elevado crescimento vegetativo para as três cultivares.

Assim, a presença de *tipburn* pode estar associada a limitações no transporte do Ca para a parte aérea em períodos de crescimento ativo. Aparentemente o fornecimento de concentrações Ca mais altas não influenciou a qualidade do fruto.

Figura 2.14: Aspeto dos ensaios com diferentes níveis de Ca. **A:** sistema de rega localizada e **B:** o Ca foi adicionado por pulverização foliar.

- ***Ensaio da estabilidade de diferentes variedades de azeite* durante o armazenamento:**

Na perspetiva do consumidor é importante que a qualidade do azeite permaneça durante o período de armazenamento. Assim, neste ensaio colaborei na realização de análises de qualidade em diferentes variedades de azeite. Como análises químicas destacaram-se a determinação da acidez, o índice de peróxidos, a p-anisidina, a quantificação dos fenóis totais e tocoferóis. Os resultados indicaram que o armazenamento dos azeites não teve efeito sobre o perfil de ácidos gordos, no entanto, a acidez aumentou rapidamente bem como o índice de peróxido e o valor de p-anisidina.

Contrariamente, os tocoferóis, os fenóis e a atividade antioxidante diminuíram com o tempo. Em algumas variedades de azeite houve uma diminuição do sabor doce e um aumento de sabor a ranço.

- ***Ensaio de indução de sintomas de micronutrientes em hortícolas:***

Colaborei ainda na instalação de um ensaio cujo objetivo geral foi o de estudar o efeito da ausência de diversos micronutrientes na atividade radicular da enzima QF-R em hortícolas (Figura 2.15), onde registei que o Manganês (Mn) e o Boro (B) atuam de modo semelhante ao Fe. enquanto

os outros micronutrientes não parecem afetar de modo direto esta atividade enzimática. Na modalidade sem Mn (-Mn) também foi possível a observação de clorose nas folhas novas inicialmente, mas em forma de manchas distribuídas irregularmente entre as nervuras das folhas e mais acentuadas nos bordos das folhas poucos dias após transplante.

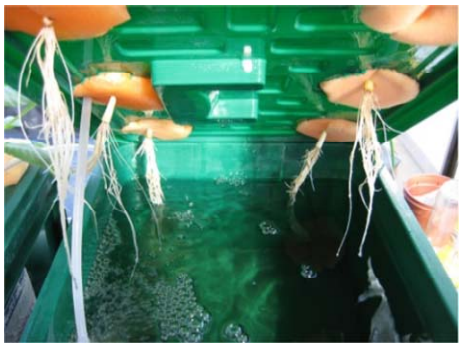
Nas plantas da modalidade sem Boro (-B), não houve alteração nos valores de SPAD, no entanto, as folhas tornaram-se quebradiças ocorrendo rotura das mesmas. Foi possível constatar os primeiros sintomas desencadeados, pela omissão de determinado micronutriente na solução nutritiva, foi o Fe e em seguida o Mn e o B, indicando a maior exigência nestes micronutrientes nas culturas em estudo.

(- Mn) Couve|folha

(- Mn) Pepino | parte aérea

(- Mn) Pepino| raiz

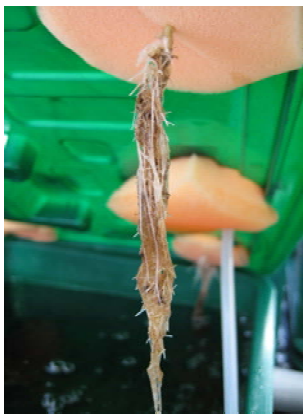
(- B) Cebola| raiz



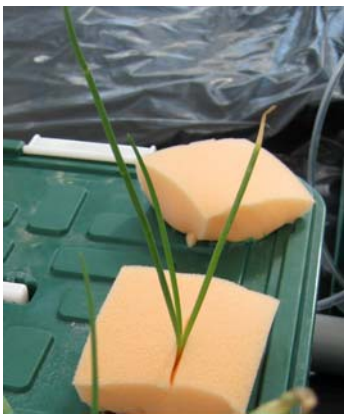
(- B) Cebola| parte aérea

(- Cu) Couve | parte aérea

(- Cu) Couve | raiz



(- Mo) Cebola| parte aérea



(- Mo) Cebola| raiz

(-Zn) Tomateiro| parte aérea (-Zn) Tomateiro| raiz

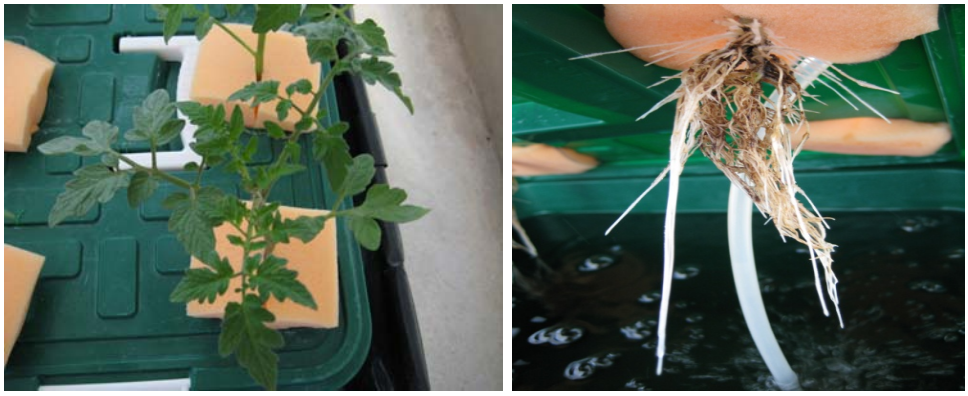


Figura 2.15: Alguns exemplos do ensaio realizado com diversas hortícolas.

- ***Comparação da qualidade de laranjas provenientes de solos calcários e não calcários:***

O objetivo deste trabalho foi o de avaliar a qualidade de laranjas, com idêntico calibre, recolhidas de pomares da cultivar 'Lanelate', estabelecidos em solo calcário e não calcário (Figura 2.16). Efetuei em laboratório a determinação da clorofila das folhas novas através do aparelho SPAD-502, bem como a partição da biomassa, através dos pesos registados na casca e na polpa. Colaborei na avaliação da qualidade das laranjas designadamente o peso fresco, o diâmetro, a cor externa, o volume e peso total de sumo, o teor de sólidos solúveis e a acidez.

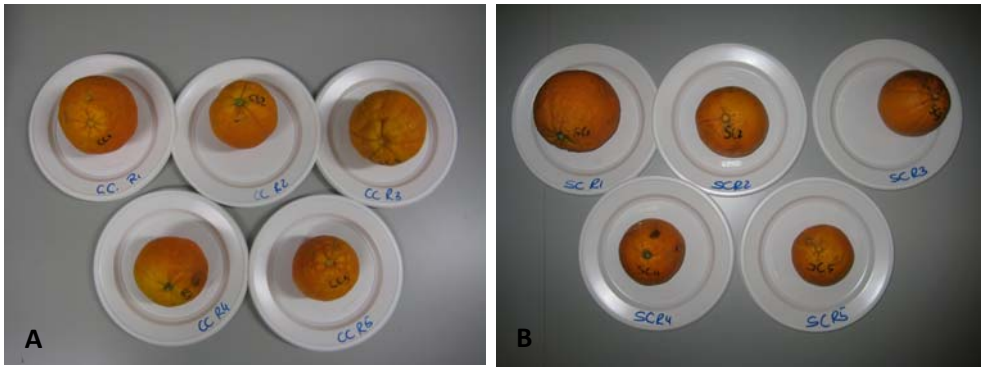


Figura 2.16: Laranjas de pomares estabelecidos em solo calcário **(A)** e em solo não calcário **(B)**.

Com os resultados obtidos neste ensaio comprovou-se os efeitos indutores do calcário na clorose férrica e o seu efeito na qualidade do fruto, que foi inferior nos frutos dos solos calcários.

- ***Recurso a modelos de Vis/NIR para a previsão dos atributos de qualidade/maturação de morango:***

Colaborei ainda na instalação de dois ensaios com diferentes cultivares de morangueiro em cultivo sem solo (‘Antilha’ e ‘Primori’), bem como na preparação de soluções nutritivas para a fertirrega. Acompanhei as diferentes modalidades em estudo e efetuei o registo semanal dos parâmetros selecionados, bem como, todas as práticas culturais necessárias à cultura incluindo as colheitas semanais e análise nutricional das plantas e frutos. Avaliei a qualidade dos frutos ao longo do ensaio, através de medições não invasivas, utilizando o aparelho Vis/NIR e através de medições destrutivas de acordo com os métodos padrão. Este projeto ainda está em curso, pelo que não foram finalizados os modelos de previsão da maturação dos morangos.

4. CONCLUSÕES TÉCNICAS

Durante o período em que trabalhei no Laboratório de Nutrição Vegetal da FCT na UALG aprendi novas metodologias de cultivo, de manutenção das culturas e diversos protocolos laboratoriais. Por outro lado, realço ainda que além de ter melhorado a minha formação e competências, os resultados provenientes dos ensaios em que participei foram publicados em revistas científicas de elevado impacto e, pela aplicação técnica que têm, contribuem para a melhoria do sector hortofrutícola.

Neste contexto é possível destacar como principais conclusões científicas e técnicas:

1. A deficiência de Fe em plantas de morangueiro provoca decréscimos no teor de clorofila e induz sintomas característicos, clorose internervuras das folhas mais jovens. Os acréscimos na atividade radicular da enzima QF-R e alterações morfológicas ao nível da raiz, são mecanismos de resposta que apesar de presentes em algumas modalidades, nem sempre foram eficientes.
2. Quanto à recuperação dos sintomas de deficiência de Fe, foi possível verificar que mesmo na ausência total de clorofila, as plantas de morangueiro conseguem reverdecer, isto é, o crescimento vegetativo é afetado mas o metabolismo é retomado após a adição de Fe pela raiz, seja na forma de quelato, seja na forma de sulfato.
3. Por outro lado, a aplicação foliar de Fe, na forma de $FeSO_4$, mostrou não ser tão eficiente mas desativou os mecanismos de resposta radicular, o que possibilitou equacionar uma nova hipótese sobre os diferentes sinais que podem existir em plantas com *stress* nutritivo. Nestas condições experimentais a atividade da enzima QF-R parece ser desativada por pulsos de Fe aplicado por pulverização foliar. Ao contrário, este mecanismo de desativação é mais lento quando Fe é aplicado diretamente para as raízes.
4. Adicionalmente, a baixa mobilidade do Fe aplicado foliarmente foi evidenciada, destacando os cuidados a ter nas pulverizações foliares e a necessidade de recorrer a molhantes para que o tratamento permaneça na superfície foliar a tratar. A aplicação foliar em certas zonas da folha foi particularmente efetiva na superfície destas e o reverdecimento foi acentuado mas confinado ao local da aplicação.
5. A utilização de extratos vegetais poderá constituir um método alternativo e inovador de correção da clorose férrica através da aplicação foliar, e estão em curso ensaios de validação agronómica com a ADP- adubos de Portugal, com vista à potencial comercialização de novos fertilizantes.

6. A alfarrobeira tem um padrão de crescimento lento o que parece contribuir para a tolerância desta espécie a uma baixa disponibilidade de Fe a nível radicular. O mesmo não ocorre com o porta-enxerto *Poncirus trifoliata* L. que, quando submetido a baixos níveis de Fe ou na ausência deste elemento apresenta uma redução significativa no seu crescimento vegetativo e nas leituras de SPAD.

7. Os resultados obtidos nestes ensaios possibilitaram ainda novas linhas de investigação integradas em um novo projeto *do* Laboratório de Nutrição Vegetal, financiado pela FCT e que tem inicio previsto em Abril deste ano.

5. REFERÊNCIAS BIBLIOGRÁFICAS

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III. *CURRÍCULUM VITAE* DETALHADO

1. SÍNTESE BIOGRÁFICA

Teresa Maria Rego Saavedra, filha de Abílio Saavedra Antunes e de Maria José Batista Rego, nasceu em Lisboa a 4 de agosto de 1982, reside atualmente em Faro. Frequentou o 1º ciclo do ensino básico de 1989 a 1992, o 2º ciclo do ensino básico de 1992 a 1997 e concluiu o 3º ciclo do ensino básico no ano letivo de 2000 com a classificação final de 15 valores. Concluiu a licenciatura em Engenharia Agronómica – Ramo Hortofruticultura em 2008 pela Universidade do Algarve (UALG) e realizou o seu estágio de fim de curso no Laboratório de Nutrição Vegetal da UALG, mediante a orientação da Professora Maribela Pestana, no qual obteve a classificação de 18 valores. Face ao mérito científico do seu trabalho de estágio, os resultados foram publicados numa revista internacional com elevado impacto na área das ciências agrárias (*Plant Physiology and Biochemistry*).

A sua atividade científica tem vindo a ser desenvolvida principalmente na área da Nutrição Vegetal e da fertilização, especificamente na clorose férrica, pela participação em projetos de investigação nacionais, da qual resultaram diversas publicações de caráter técnico e científico. Nos últimos anos mantem-se no laboratório de Nutrição Vegetal a executar com rigor as tarefas que lhe são afetas. A candidata apresenta um perfil bastante diversificado pois já realizou ensaios em campo, em vaso e em hidroponia (com recurso a diferentes sistemas), tendo trabalhado com as mais diversas espécies nomeadamente morangueiro, alfarrobeira, porta-enxertos de citrinos e laranjeiras.

Nestes trabalhos foi responsável pelas práticas culturais relacionadas e com o ciclo produtivo de cada uma das espécies. Realizou também análises da composição mineral de material vegetal, frutos, bem como da qualidade organolética e comercial de frutos. Testou novos fertilizantes para a empresa ADP-Adubos de Portugal S.A. (ADP). Além disso, apresenta diversas competências laborais.

Durante este período teve a oportunidade de coorientar a parte prática de alguns estágios de licenciatura. No âmbito da sua atividade profissional tem vindo a desenvolver várias competências, conhecimentos relevantes, experiência prática e sentido de responsabilidade, para além de contactar diretamente com produtores, técnicos e investigadores do setor. Destaca-se ainda a experiência que obteve na utilização de programas de estatística, nomeadamente SPSS, e na elaboração de relatórios técnicos sendo coautora de artigos científicos, bem como, apresentou vários trabalhos científicos oralmente ou por painel, em simpósios nacionais e internacionais. Deste modo o grau de mestre em Hortofruticultura permitir-lhe-á dar continuidade ao trabalho que tem vindo a desenvolver nesta Universidade, bem como fomentar e aprofundar de forma específica os seus conhecimentos e concomitantemente, aplica-los no exercício das suas funções.

DADOS BIOGRÁFICOS

Nome: Teresa Maria Rego Saavedra

Citação: T. Saavedra

Data e local de nascimento: 04 de agosto de 1982, Lisboa.

Nacionalidade: Portuguesa

Estado civil: Solteira

Bilhete de identidade: 12038147

Carta de condução: Obtida a 30/08/2004.

Morada: Urbanização Pinhal da Ria lote 7, 2º esquerdo, Montenegro, 8005- 175 Faro.

Telemóvel: 919443434

E- mail: tmr.saavedra@gmail.com

2. COMPETÊNCIAS PESSOAIS

Pessoais: Pontual, dinâmica, empenhada e responsável. Gosto por trabalho em equipa

Informática: Domínio na utilização do Microsoft Office e do programa de estatística - SPSS (Statistical Package for Social Sciences) na análise de variância, comparação múltipla de médias e regressão linear.

Línguas: Bons conhecimentos de Inglês escrito e falado.

3. HABILITAÇÕES ACADÉMICAS

Julho 2008 Concluiu a licenciatura em Engenharia Agronómica – ramo Hortofruticultura pela Universidade do Algarve, com nota final de 12 valores.

Julho 2008 Apresentou o trabalho de estágio realizado na Universidade do Algarve intitulado "*Estudo da clorose férrica em plantas de morangueiro (Fragaria x ananassa duch.)* ", com a classificação final de 18 valores.

Julho 2000 Concluiu o Ensino Secundário, Agrupamento 1 – Cientifico ou natural com média final de 15 valores.

4. ATIVIDADE PROFISSIONAL

De dezembro de 2012 até ao presente é bolseira de investigação no âmbito do Vale de Inovação e respetivo contrato de prestação de serviços estabelecido entre a empresa Dandlen & Vasquez, Lda e a UALG. Realiza ensaios mediante coordenação da Professora Maribela Pestana.

O acordo de confidencialidade estabelecido entre as duas partes impede a divulgação dos trabalhos em curso.

De dezembro 2011 a julho 2012 foi bolseira de investigação no âmbito do projeto de investigação I2TEP, na UALG, mediante coordenação do Professor Pedro José Correia.

As principais atividades desenvolvidas neste projeto foram a instalação de dois ensaios com diferentes cultivares de morangueiro em cultivo sem solo, bem como a preparação de soluções nutritivas para a fertirrega. Acompanhou diferentes modalidades em estudo: efetuou o registo semanal dos parâmetros selecionados, bem como, todas as práticas culturais necessárias à cultura incluindo as colheitas semanais e análise nutricional das plantas e frutos. Avaliou a qualidade dos frutos através de medições não invasivas, utilizando o aparelho Vis/NIR e determinou valores de firmeza, º Brix e acidez titulável (medições destrutivas) de acordo com os métodos padrão. Participou na preparação de artigos científicos resultantes. Os principais objetivos foram otimizar os *inputs* nutricionais para a cultura do morangueiro.

De julho 2011 a setembro 2011 foi bolseira de investigação no âmbito do Acordo Específico de Licenciamento Exclusivo de Tecnologia entre a UALG e a ADP-Adubos de Portugal**,** mediante coordenação da Professora Doutora Maribela Pestana.

No âmbito da bolsa foram instalados e acompanhados ensaios de validação agronómica em vaso, de novos fertilizantes líquidos com Fe aplicados por pulverização foliar. Preparou o substrato com adubação de fundo e fez o transplante das plantas, tomate para indústria, com instalação de rega automática. Avaliou o impacto dos novos fertilizantes no equilíbrio nutricional da produção. Realizou a análise estatística dos resultados obtidos que foram integrados no respetivo relatório final.

De fevereiro 2011 a junho 2011 foi colaboradora em regime de prestação de serviços no âmbito do projeto PTDC/AGR-AAM/100115/2008 "A estratégia nutricional da alfarrobeira nos solos calcários desenvolvido na UALG, mediante coordenação do Professor Pedro José Correia.

Desenvolveu as seguintes análises: composição mineral de folhas e ramos, quantificação da atividade de enzimas envolvidas no metabolismo do Fe e preparou as soluções nutritivas para a instalação de ensaios com citrinos e alfarrobeiras. Fez a sementeira destas plantas em substrato inerte (vermiculite).

De setembro 2010 a janeiro 2011 foi bolseira de investigação no âmbito do Acordo Específico de Licenciamento Exclusivo de Tecnologia entre a UALG e a ADP-Adubos de Portugal, mediante coordenação da Professora Maribela Pestana.

Estabeleceu e acompanhou os ensaios de validação agronómica, em vaso e em culturas sem solo, avaliou a dissolução e a estabilidade de diferentes fontes de Fe, que não pode divulgar devido ao compromisso de sigilo com a empresa. Quantificou o teor de Fe e testou a eficácia agronómica das formulações mais adequadas em plantas com clorose férrica.

De dezembro 2009 a setembro 2010 foi colaboradora no âmbito do projeto em curso *New approaches in the characterization and treatment of iron chlorosis. Iron fluxes, carriers, and gene expression* (PTDC/AGR-ALI/66065/2006 – NAICE) da UALG, mediante coordenação das Professoras Maribela Pestana e Maria da Graça Miguel.

Como atividades desenvolvidas destacam-se: quantificou a atividade enzimática da QF-R e o teor mineral das plantas por absorção atómica-EAA. Preparou as amostras e colaborou no procedimento de quantificação e identificação do teor de ácidos orgânicos envolvidos no transporte do Fe na planta (por cromatografia-HPLC). Avaliou os efeitos da clorose férrica no teor de ácidos orgânicos e na atividade antioxidante de frutos. Participou na análise estatística dos dados obtidos.

De março 2009 a novembro 2009 foi bolseira de Investigação da UALG no âmbito do Prémio Caixa de Crédito Agrícola 2008 "Uso de aparas de relvas como fertilizante", coordenado pela Professora Maribela Pestana.

As atividades desenvolvidas no âmbito da bolsa foram estabelecer e acompanhar ensaios de validação agronómica com tratamentos alternativos de correção da deficiência de Fe. Deste modo, preparou e testou um extrato vegetal a partir das aparas de relvas e testou a sua aplicação (patente nacional da UALG-103584 e internacional da UALG em copropriedade com a ADP-Adubos de Portugal -PCT/PT2007/000041). Realizou a análise estatística dos resultados obtidos, integrados no respetivo relatório.

De abril 2008 a fevereiro 2009 efetuou várias análises químicas, em regime de prestação de serviços no Centro de Desenvolvimento de Ciências e Técnicas de Produção Vegetal (CDCTPV) da UALG, orientada pela Professora Maria Graça Miguel.

Realizou análises de qualidade em azeites. Como métodos destacam-se: a determinação da acidez, índice de peróxidos,p- anisidina, quantificação dos fenóis totais e tocoferóis nos azeites estudados.

De outubro 2007 a abril 2008 colaborou em regime de prestação de serviços no projeto Agroambiente do CDCTPV da UALG, mediante coordenação do Professor Pedro José Correia.

Como tarefas destacaram-se a instalação de um ensaio com cultivares de morangueiro em cultivo sem solo. Instalou e acompanhou as diferentes modalidades em estudo. Efetuou o registo semanal dos dados, bem como, todas as práticas culturais necessárias incluindo as colheitas semanais. Avaliou o efeito de diferentes concentrações de Ca na incidência do *tipburn* no crescimento vegetativo nos parâmetros de qualidade do fruto.

De março 2007 a julho 2007 realizou trabalho de campo e análises laboratoriais no CDCTPV da UALG, mediante coordenação da Professora Maribela Pestana.

Como tarefas destacaram-se a instalação de um ensaio de uma cultivar de morangueiro em hidroponia, o registo semanal dos dados referentes a parâmetros de crescimento vegetativo e parâmetros de biomassa. Efetuou a determinação da atividade enzimática da QF-R, envolvidas no metabolismo do Fe.

5. COLABORAÇÃO NA ORIENTAÇÃO PRÁTICA

Colaborou na orientação laboratorial e de campo dos seguintes trabalhos finais de fim de curso que decorreram no Laboratório de Nutrição Vegetal da UALG:

De junho 2009 a dezembro 2009 - Colaborou na orientação da parte prática do estágio curricular da licenciatura em Engenharia Agronómica da aluna Dora Lopes "O uso de resíduos minerais no fabrico de fertilizantes líquidos com Fe" realizado na UALG/FCT, orientado pela Professora Maribela Pestana Correia.

De janeiro 2009 a junho 2009 Colaborou na orientação laboratorial e de campo do aluno Pedro Cupertino, Bolseiro de Iniciação à Investigação (BII) da FCT, realizado no CDCTPV da UALG orientado pelo Professor Pedro Correia.

De outubro 2007 a abril 2008 Colaborou na orientação laboratorial e de campo do estágio curricular da licenciatura em Engenharia Agronómica do aluno Edelberto Ribeiro, "Efeito do Ca na incidência *Tipburn* e no desenvolvimento de três variedades de morangueiro", realizado na UALG e orientado pelo Professor Pedro Correia.

6. PARTICIPAÇÃO E COMPARÊNCIA A CONGRESSOS

6.1.PARTICIPAÇÃO EM CONGRESSOS: COMUNICAÇÕES ORAIS (CO) OU PAINÉIS (P)

P1. P.J. Correia, D. Lopes, A. Duarte, F. Gama, **T. Saavedra**, M. Pestana **(2012)** Is there a relationship between ferric-chelate reductase activity in roots of *Poncirus trifoliata* and leaf chlorophyll contents? XII International Citrus Congress, Valencia, Espanha, (18-23 nov) p. 329 **(P)**.

O objetivo deste estudo foi investigar a atividade da enzima QF-R e estabelecer uma relação com a clorofila ou grau clorose das plantas de *Poncirus trifoliata*. Participou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos dados e determinou a atividade da enzima QF-R, bem como os vários parâmetros de biomassa. Como conclusões deste trabalho, destaca-se: plantas que cresceram sem Fe, manifestaram clorose nas folhas, mas nas condições experimentais do ensaio, o crescimento da parte aérea não foi afetado. Os valores mais elevados da atividade da QF-R relacionaram-se com os valores de SPAD, o que indica uma resposta a condições de *stress*.

CO1. M. Pestana, F. Gama, **T. Saavedra**, J. Castro Pinto, A. Abadía, A. de Varennes, P.J. Correia **(2012)** A caraterização e correção da deficiência de Fe em plantas de morangueiro: novas abordagens. Livro de resumos do *IV Colóquio Nacional da Produção de Pequenos Frutos*, APH, Faro, p. 11. **(CO)**

O objetivo geral desta comunicação foi apresentar de forma resumida os resultados obtidos em diversos ensaios com plantas de morangueiro (*Fragaria × ananassa* Duch.). Como objetivos específicos destacam-se: o estudo dos mecanismos fisiológicos e bioquímicos de controlo da deficiência de Fe e a avaliação de novas alternativas para a correção da clorose férrica. Através dos resultados obtidos constatou-se que é possível otimizar a época de aplicação do Fe e desenvolver novos métodos que permitam dinamizar as reservas nativas de Fe na planta.

P2. M. Pestana, F. Gama, **T. Saavedra**, H. El-Jendoubi, P.J. Correia, A. Abadía **(2012)** A mobilidade do Fe nas folhas de morangueiros cloróticos. Livro de resumos do *IV Colóquio Nacional da Produção de Pequenos Frutos*, APH, Faro, p. 13. **(P)**

O objetivo deste trabalho foi o de estudar a mobilidade do Fe em folhas cloróticas de morangueiros, quando aplicado pontualmente em diferentes zonas da folha. Participou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos dados, bem como, as aplicações com $FeSO_4$. Foi concluído que a aplicação do fertilizante foliar foi particularmente efetiva na superfície das folhas e o reverdecimento acentuado. Os efeitos fora das zonas não tratadas foram mínimos, sendo o efeito do Fe aplicado foliarmente muito restrito e localizado aos locais tratados.

P3. P.J. Correia, P. Palencia, F. Martinez, F. Gama, **T. Saavedra**, M. Pestana **(2012)** A termografia como técnica de diagnóstico da clorose férrica em morangueiro. Livro de resumos do *IV Colóquio Nacional da Produção de Pequenos Frutos*, APH, Faro, p. 14. **(P)**

O objetivo deste trabalho foi o de avaliar se as variações na temperatura das folhas de morangueiro obtidas por termografia refletem o grau de clorose férrica, identificado pelos valores de SPAD. Participou na instalação de um ensaio com cultivares de morangueiro em cultivo sem solo, bem como a preparação de soluções nutritivas para fertirrega. Foi possível concluir que através da termografia é possível distinguir diferentes graus de clorose férrica em morangueiro. Através da termografia será possível antecipar os pontos de *stress* com maior precisão, o que poderá possibilitar uma correção atempada.

P4. Pestana, M., Gama, F., **Saavedra, T**., Duarte, A., Abadia, A., Varennes, A. Correia P.J. **(2011)**. Estudo comparativo da resposta fisiológica de *Ceratonia silig*ua (L.) e *Poncirus trifoliata* (L.) Raf. à deficiência de Fe. XIX Reunión de la Sociedad Española de Fisiología Vegetal. XII Congreso Hispano-Luso de Fisiología Vegetal. Castelló de la Plana, Espanha **(P)**.

O objetivo deste ensaio foi de comparar a resposta fisiológica à deficiência de Fe de plantas de C*eratonia síliqua* (alfarrobeira) com *Poncirus trifoliata* (L.) Raf., porta-enxerto de citrino muito suscetível à clorose férrica. Colaborou na instalação do ensaio com as cultivares de alfarrobeira e *Poncirus trifoliata* em hidroponia, estabeleceu diferentes modalidades em estudo e determinou diversos parâmetros de crescimento. Como principais conclusões destacaram-se que nas concentrações mais baixas de Fe, as plantas de *Poncirus trifoliata* apresentaram sintomas graves de clorose férrica, contrariamente às de alfarrobeira, que apenas apresentaram um ligeiro decréscimo nos valores de SPAD nas últimas datas, evidenciando sintomas ligeiros. A atividade da QF-R foi superior nas plantas de alfarrobeira que cresceram sempre sem Fe, enquanto nas plantas de *Poncirus trifoliata* esse incremento só se manifestou na concentração mais baixa de Fe (1 μM). Estes resultados evidenciam que as respostas diferenciadas destas espécies poderão estar associadas a diferentes estratégias na indução dos mecanismos de resposta.

P5. M. Pestana, I. Domingos, F. Gama, **T. Saavedra**, J. Castro-Pinto, A. de Varennes and P.J. Correia **(2010)** A grass clippings extract to control iron chlorosis in strawberry plants. 15th International Symposium on Iron Nutrition and Interactions in Plants. Budapeste, Hungria, p. 79 **(P)**.

O objetivo deste trabalho foi avaliar a recuperação da deficiência de Fe e os efeitos sobre o crescimento das plantas de morangueiro por pulverização foliar usando um extrato de aparas de relva, um tratamento inovador e amigo do ambiente. Colaborou na instalação do ensaio em hidroponia, instalou e acompanhou ensaios de validação agronómica com o tratamento alternativo de correção da deficiência de Fe. Deste modo, elaborou o extrato a partir das aparas de relvas para posterior correção da clorose férrica, aplicado por via foliar. Ficou concluído que os resultados deste estudo proporcionaram uma nova abordagem para a correção de clorose férrica através de um tratamento inovador e amigo do ambiente. As pulverizações foliares com um extrato de aparas de relva trouxeram algumas novas pistas para compreender os processos fisiológicos relacionados com a clorose férrica em plantas de morangueiro.

P6. M.L Osório, J. Osório, F. Gama, **T. Saavedra**, P.J. Correia and M. Pestana **(2010)** Chlorophyll fluorescence imaging as a tool for evaluation of photosynthetic responses to iron deficiency and ressupply in *Fragaria × ananassa* Duch. cv 'Diamond'. XI Congresso Hispano-Luso de Fisiologia Vegetal (8-11 setembro), Saragoça, Espanha **(P)**.

O objetivo deste trabalho foi avaliar os efeitos da deficiência de Fe de plantas de morangueiro e a sua posterior recuperação, nos teores totais de clorofila e na eficiência da conversão de energia fotossintética nas folhas novas. Participou apenas na instalação do ensaio em hidroponia, bem como na preparação de soluções nutritivas.

CO2. M. Pestana, M.H. Rodrigues, A. Machado, F. Gama, **T. Saavedra**, E. Ribeiro, F. Martinez, P. Palencia and P.J. Correia **(2010)** Nova estratégia de controlo da clorose férrica em morangueiro – subprojeto Hydropon. 2º Workshop do Projeto Rise. Beja.

Os objetivos do ensaio foram otimizar e melhorar o cultivo sem solo do morangueiro, utilizando fibra de coco como substrato, controlar a deficiência de Fe através do uso de produtos alternativos aos quelatos sintéticos e avaliar o impacto destas aplicações no balanço nutricional e na qualidade dos frutos. Colaborou na instalação do ensaio da cultivar de morangueiro em cultivo sem solo, bem como a preparação de soluções nutritivas para fertirrega. Testou a eficácia agronómica das formulações dos produtos alternativos aplicados nas plantas. Através dos resultados obtidos constatou-se que é possível minimizar as perdas e desenvolver novos métodos que permitam mobilizar as reservas endógenas de Fe na planta.

CO3. M. Pestana, I. Domingos, **T. Saavedra**, F. Gama, J. Castro Pinto, A. de Varennes and P.J. Correia **(2009)** O uso de aparas de relva na correção da clorose férrica. **Encontro Anual da Sociedade Portuguesa da Ciência do Solo. Faro** (8 – 10 julho), Portugal **(apresentado por T. Saavedra)**.

O objetivo do trabalho foi estudar o efeito da pulverização foliar do extrato vegetal produzido através de aparas de relva na recuperação da clorose férrica. As atividades desenvolvidas no âmbito deste ensaio foram instalar e acompanhar o ensaio de validação agronómica com o tratamento alternativo de correção da deficiência de Fe. O Fe existente no extrato está numa forma bastante móvel o que pode dever-se ao elevado poder complexante para o Fe.

P7. P. Palencia, F. Martinez, M. Pestana, E. Ribeiro, F. Gama, **T. Saavedra**, and P.J. Correia **(2009)** Relação entre a incidência do *tipburn* no morangueiro e a concentração de Ca na solução de rega. **Encontro Anual da Sociedade Portuguesa da Ciência do Solo. Faro** (8 – 10 julho), Portugal **(P)**.

O objetivo do trabalho baseou-se em avaliar as relações entre a incidência de *tipburn* em morangueiro (*Fragaria x ananassa* Duch.) e a aplicação de Ca na solução de rega. Participou no acompanhamento do ensaio em cultivo sem solo, na preparação de soluções nutritivas para fertirrega. Instalou as diferentes modalidades em estudo. Concluiu-se que este desequilíbrio nutricional parece estar mais relacionado com o crescimento vegetativo e variação sazonal do que com a disponibilidade do Ca no substrato.

P8. T. Saavedra, F. Gama, P.J. Correia, and M. Pestana **(2009)** Deficiência de Fe em plantas de morangueiro: efeitos na partição da biomassa e na produção. **VI Congresso Ibérico de Ciências Hortícolas**. Logronho (25-29 maio), Espanha **(P)**.

Pretendeu-se estudar o efeito da deficiência de Fe, no padrão de partição da biomassa e na produção total em plantas de morangueiro (*Fragaria* x *ananassa* Duch). Participou no acompanhamento do ensaio em cultivo sem solo, na preparação de soluções nutritivas, efetuou o registo semanal dos dados, fez colheitas semanais e análise nutricional das plantas e frutos e quantificou do teor mineral das plantas sujeitas a diferentes níveis de Fe por espectrofotometria de Absorção Atómica (EAA). Concluiu-se que a clorose férrica antecipou a entrada em floração e em produção do morangueiro no primeiro ciclo, produzindo mais frutos por planta mas mais pequenos (diâmetro inferior a 18 mm - categoria III). As plantas controlo emitiram mais estolhos mas produziram menos frutos. As plantas cloróticas tiveram um investimento preferencial no crescimento reprodutivo em detrimento do vegetativo.

P9. M. Pestana, F. Gama, **T. Saavedra**, S. Dandlen, and M.G. Miguel. **(2009)** Quality of strawberry fruits (cv. 'Selva') as affected by Fe deficiency. International Conference on Environmentally Friendly and Safe Technologies for Quality of fruits and vegetables. Faro (14-16 janeiro), Portugal **(P)**.

O objetivo deste trabalho foi avaliar os efeitos da deficiência de Fe na qualidade química do sumo de morango. Colaborou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos dados. Determinou a qualidade da produção como: determinação do total de sólidos solúveis (º Brix), da cor externa e da firmeza de frutos, atividades antioxidantes em frutos nomeadamente, DPPH (Free Radical Scavenging Activity), e a concentração total de fenóis. Verificou-se uma forte associação entre a concentração de ácido ascórbico e o poder redutor. A razão de malato/citrato aumenta com a deficiência de Fe, indicando um possível atraso na maturação do fruto.

P10. M. Pestana, F. Gama, **T. Saavedra**, S. Dandlen and M.G. Miguel **(2008)** The effects of Fe deficiency on organic acids, sugars and anthocyanins in strawberry fruits. XII Simpósio Ibérico de Nutrição Mineral de Plantas. Granada (22-24 outubro), Espanha **(P)**.

O objetivo do estudo foi o de avaliar o efeito da deficiência de Fe nas características químicas dos morangos colhidos de plantas desenvolvidas com diferentes concentrações de Fe na solução nutritiva. Colaborou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos dados. Determinou a qualidade da produção como: determinação do total de sólidos solúveis (º Brix), da cor externa e da firmeza de frutos, atividades antioxidantes em frutos nomeadamente, DPPH (Free Radical Scavenging Activity), concentração total de fenóis, ácidos orgânicos, antocianinas e açúcares. Morangueiros com sintomas de clorose férrica produzem frutos com peso semelhante, mas com menor intensidade de cor e pobres em características organoléticas. Como frutos não climatéricos, o desequilíbrio nutricional induzido pela deficiência de Fe pode afetar não apenas a data da colheita, mas também o armazenamento e subsequente comercialização.

P11. Gama, S. Dandlen, T. Saavedra, M. Pestana and M.G. Miguel **(2008)** Evaluation of Fe deficiency effects on antioxidant activity of strawberry fruits. VI International ISHS Symposium on Mineral Nutrition of Fruit Crops. Faro (19 a 23 maio), Portugal **(P)**.

O objetivo deste trabalho foi avaliar os efeitos da deficiência de Fe sobre a atividade antioxidante do morango. Colaborou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos dados. Concluiu-se que o elevado teor em ácido ascórbico parece ter sido o responsável pela atividade antioxidante elevada, que está relacionada com a atividade TEAC e não com os compostos de fenólicos.

P12. M. Pestana, F. Gama, T. Saavedra, S. Dandlen and M.G. Miguel **(2008)** Effects of Fe deficiency on strawberry (cv. 'Selva') fruit quality. VI Internacional Strawberry Symposium. Huelva (3-7 março), Espanha **(P)**.

O objetivo deste trabalho foi avaliar os efeitos da deficiência de Fe na qualidade química do morango. Colaborou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos dados. Determinou a qualidade da produção. A razão de malato/citrato aumentou com a deficiência de Fe, indicando um atraso na maturação do fruto.

6.2.COMPARÊNCIA EM CONGRESSOS, WORKSHOPS E FORMAÇÕES

Fevereiro 2010 Assistiu ao Seminário "Caracterização da clorose férrica em plantas de morangueiro (Fragaria x ananassa Duch. Cv. Diamante)", realizada no CDCTPV na UALG no 17 de fevereiro.

Maio 2009 Assistiu ao Seminário "Alternativas químicas para la desinfeccion del suelo en el cultivo de la fresa" e "Control biologico de enfermedades de fresa en sistemas de cultivo sin suelo", produzido pelos Professores pedro Palência e Fátima Martinez da Universidade de Huelva, organizado pelo CDCTPV, na UALG no dia 18 maio.

Abril 2009 Participou, como monitora, na iniciativa "*Encontros Imediatos com a Ciência*" realizada de 27 abril a 30 de abril, no Centro de Ciência Viva de Tavira.

Novembro 2008 Assistiu ao "I Colóquio de Agricultura Biológica do Algarve", realizado na Direção Regional de Agricultura e Pescas do Algarve, Patacão – Faro, de 18-19 de novembro.

Julho 2008 Assistiu ao *III Congresso Ibérico da Ciência do Solo*, que decorreu de 1 a 4 de julho de 2008 em Évora.

Julho 2008 Participou no *1º Workshop Inovações na Agricultura – Determinação Não Destrutiva da Qualidade de Frutos*, realizada no 23 de julho na UALG.

Abril 2008 Participou, como monitora, na iniciativa "*Encontros Imediatos com a Ciência*" realizada de 31 de março a 4 de abril, no Centro de Ciência Viva de Tavira.

Fevereiro 2008 Assistiu ao Seminário Técnico "Uso eficiente da água", organizado pela Almargem e UALG, Faro.

Fevereiro 2008 Assistiu ao 2º Congresso Nacional de Citricultura que decorreu de 24 a 26 de janeiro de 2008 em Faro.

Maio 2006 Assistiu ao Seminário **"**Tradicional food processing and technological innovation in the peripheral regions", que decorreu a 26 de maio de 2006 na UALG, Faro.

Novembro 2006 Assistiu ao Seminário "Estratégias para o desenvolvimento rural 2007-2013 - Oportunidades e ameaças para o Algarve", que decorreu a 23 de novembro de 2006, na UALG, Faro.

7. COMPETÊNCIAS TÉCNICO- CIENTÍFICAS

No âmbito da sua atividade científica, como bolseira e/ou colaboradora de diversos projetos da UALG, tem experiência em ensaios de campo, em vaso e em hidroponia, complementada pelo domínio de diversos metodologias analíticas, e respetivos equipamentos.

Destacam-se os seguintes procedimentos associados ao delineamento, instalação e acompanhamento de **ensaios**:

No campo e em vaso, na instalação e acompanhamento de ensaios para testar novos fertilizantes líquidos com Fe realizados em pomares de citrinos. Em estufa e em hidroponia, designadamente em solução nutritiva e em placas de polietileno com substrato inerte. Culturas instaladas e acompanhadas: morangueiro, tomateiro, pimenteiro, cebola, pepino, entre outras.

Como **metodologias laboratoriais** destacam-se:

A Espectrofotometria de Absorção Atómica (EAA) na determinação da composição mineral de folhas, flores e frutos.

A Espectrofotometria de Absorção Molecular (colorimetria) para a determinação da atividade enzimática da QF-R em ápices radiculares.

A identificação e quantificação de ácidos orgânicos em material vegetal por HPLC (High Performance Liquid Chromatography) e LCMS (Liquid Chromatography – Mass Spectrometry) pelo método de diluição de isótopos.

A utilização do refratómetro, do texturómetro e do aparelho Vis/NIR para a avaliação da qualidade da produção como: determinação do total de sólidos solúveis (º Brix), da cor externa e da firmeza de frutos.

As atividades antioxidantes em frutos nomeadamente, DPPH (Free Radical Scavenging Activity), TEAC (Trolox Equivalent Antioxidant Capacity, ORAC (Oxygen Radical Absorbance Capacity) e Poder Redutor.

A caracterização e quantificação de proteínas em material vegetal. Método de Bradford e análise qualitativa de proteínas por Eletroforese SDS- PAGE e potenciometria.

8. FILIAÇÕES EM ASSOCIAÇÕES CIENTÍFICAS

2009 – até ao presente Sócio da Sociedade Portuguesa da Ciência do Solo (SPCS).

2009 – até ao presente Sócio da Sociedade Portuguesa de Bioquímica, com filiação à Sociedade Portuguesa de Fisiologia vegetal.

9. OUTRAS ATIVIDADES

Julho 2009 Colaborou junto da Comissão Organizadora no Encontro Anual da Sociedade Portuguesa da Ciência do Solo, realizado na Universidade do Algarve, Faro, Portugal.

Janeiro 2009 Colaborou junto da Comissão Organizadora da Conferência Internacional, *Environmentally Friendly and Safe Techonologies for Quality of Fruits and Vegetables* que decorreu na Universidade do Algarve, Faro, Portugal.

Abril 2009 Participou, como monitora, na iniciativa "*Encontros Imediatos com a Ciência*" realizada de 27 abril a 30 de abril, no Centro de Ciência Viva de Tavira

Maio 2008 Colaborou junto da Comissão Organizadora do *VI International ISHS Symposium on Mineral Nutrition of Fruit Crops.* Faro, Portugal.

Abril 2008 Participou, como monitora do CDCTPV, na iniciativa "*Encontros Imediatos com a Ciência*" realizada de 31 de março a 4 de abril, no Centro de Ciência Viva de Tavira.

10. PUBLICAÇÕES

Da atividade científica desenvolvida resultaram diversas publicações que estão publicadas em revistas internacionais com arbitragem científica. Também participou em diversos simpósios nacionais e internacionais de que resultaram diversas publicações nos respetivos livros de atas.

10.1. RELATÓRIOS

T. Saavedra (2007) Estudo da clorose férrica em plantas de morangueiro (*Fragaria x ananassa Duch*). Tese de relatório de estágio do curso de licenciatura em Engenharia Agronómica – ramo Hortofruticultura, Universidade do Algarve, 48 p.

O objetivo geral deste trabalho foi o de caracterizar a deficiência de Fe e a posterior comparação da recuperação dos sintomas de clorose férrica pela adição de $FeSO_4$ à solução ou por pulverização foliar a plantas de morangueiro. Executou todo o trabalho experimental. A deficiência de Fe motivou acréscimos na atividade da QF-R e, pelo contrário, níveis elevados de Fe no meio inibiram a atividade desta enzima. A presença de sintomas de clorose férrica provocou uma antecipação da entrada em produção, contudo diminuiu a qualidade de produção obtida. Verificou-se ainda que os morangueiros conseguem recuperar após um período de crescimento sem Fe; no entanto, o facto do crescimento vegetativo ter sido afetado já não é possível que recupere no período em estudo. Os efeitos do reverdecimento foram mais acentuados nas plantas em que foi adicionado $FeSO_4$ na solução nutritiva. A recuperação por via foliar foi mais limitada, o que poderá ter a ver com a forma de Fe aplicada.

10.2 ARTIGOS SUBMETIDOS (AS) OU PUBLICADOS (A) EM REVISTAS INTERNACIONAIS COM ARBITRAGEM CIENTÍFICA

AS1. P.J. Correia, A. de Varennes, F. Gama, **T. Saavedra**, M. Pestana **(2013)** Nutritional partition in *Poncirus trifoliata* and *Ceratonia siliqua* grown under different concentrations of iron in nutrient solution. *Journal of Plant Nutrition and Soil Science*. ***Submetido*** ao Journal of Plant Nutrition and Soil Science.

AS2. T. Saavedra, M.D. Antunes, S. A. Dandlen, M.A. Neves, D. Martins, A.C. Figueiredo, L.G. Pedro, J.G. Barroso, M.G. Miguel*,* **(2012)** Stability and sensory characteristics of olive oils during storage in the presence of *Thymbra capitata* essential oil. ***Submetido*** ao International Journal of Food Properties

A1. M. Pestana, P.J. Correia, **T. Saavedra**, F. Gama, S. Dandlen, G. Nolasco ; A. de Varennes **(2012)** The root ferric chelate reductase can be regulated by iron and copper in shoots. Journal of Plant Nutrition. ***in press***.

Neste trabalho o objetivo foi estudar a interação entre o Cu e o Fe em plantas de morangueiro cultivadas em soluções nutritivas com diferentes concentrações de Fe. Participou na instalação do ensaio em hidroponia de plantas de morangueiro, preparou as soluções nutritivas e efetuou o registo semanal dos parâmetros de crescimento vegetativo e de biomassa. Neste trabalho concluiu-se que as raízes das plantas que cresceram sem Fe eram menores e menos ramificadas. Em comparação com outros tratamentos, a concentração de Cu nas raízes das plantas que cresceram sem Fe foi três vezes superior, indicando que o Cu pode ter sido absorvido em substituição do Fe. Como a atividade radicular da enzima QF-R foi induzida pela ausência de Fe na solução nutritiva, não havia Fe para ser reduzido, sendo substituído pelo Cu, que após redução pela QF-R foi absorvido.

A2. M. Pestana, P.J. Correia, **T. Saavedra,** F. Gama, A. Abadía; A. de Varennes **(2012)** Development and recovery of iron deficiency by iron resupply to roots or leaves of strawberry plants. Plant Physiology and Biochemistry. 53: 1-5.

doi.org/10.1016/j.plaphy.2012.01.001

Este ensaio teve como objetivo estudar a deficiência de Fe em morangueiro (*Fragaria x ananassa* Duch) e avaliar a capacidade de recuperação comparando a aplicação de $FeSO_4$ à solução nutritiva e por pulverização foliar. Colaborou na instalação do ensaio de plantas de morangueiro em cultivo sem solo. Preparou soluções nutritivas para hidroponia. Estabeleceu diferentes modalidades em estudo, bem como, todas as aplicações feitas com $FeSO_4$. Determinou a atividade enzimática QF-R envolvida no metabolismo do Fe. Como conclusões deste trabalho foi possível destacar que as plantas de morangueiro que cresceram sempre sem Fe apresentaram sintomas de clorose férrica e alterações da morfologia externa das raízes, acompanhadas por incrementos na atividade radicular da QF-R. Nas plantas recuperadas pela aplicação de Fe à solução, a atividade da QF-R manteve-se alta, sugerindo uma estratégia que pode ser usada para incrementar as reservas deste elemento.

A3. M. Pestana; F. Gama; **T. Saavedra**; A. de Varennes, P.J. Correia **(2012)** The root ferric-chelate reductase of *Ceratonia siliqua* (L.) and *Poncirus trifoliata* (L.) Raf. respond differently to levels of iron. *Scientia Horticulturae*. 135: 65-67.

doi:10.1016/j.scienta.2011.12.018

O objetivo deste ensaio foi de comparar a resposta fisiológica à deficiência Fe de alfarrobeiras (*Ceratonia síliqua* L.) com a do *Poncirus trifoliata* L., um porta-enxerto de citrinos muito suscetível à clorose férrica. Colaborou na instalação do ensaio, instalou as diferentes modalidades em estudo e determinou diversos parâmetros de crescimento. Como principais conclusões destacam-se: nas concentrações mais baixas de Fe, as plantas de *Poncirus trifoliata* L. apresentaram sintomas graves de clorose férrica, contrariamente às de alfarrobeira, que apenas apresentaram nas últimas datas sintomas ligeiros de clorose férrica. A atividade da QF-R foi superior nas plantas de alfarrobeira que cresceram sempre sem Fe, enquanto nas plantas de *Poncirus trifoliata* L. esse incremento só se manifestou na concentração mais baixa de Fe (1 μM). Estes resultados evidenciam que as respostas diferenciadas destas espécies poderão estar associadas a diferentes estratégias na indução dos mecanismos de resposta.

A4. P.J. Correia, M. Pestana, F. Martinez, E. Ribeiro, F. Gama, **T. Saavedra**, P. Palencia **(2011)** Relationships between strawberry fruit quality attributes and crop load. *Scientia Horticulturae*, 130: 398-403.

doi:10.1016/j.scienta.2011.06.039

Pretendeu-se estudar o efeito de diferentes concentrações de Ca aplicada na forma de nitrato de cálcio ($CaNO_3$), na qualidade de plantas de morangueiro (*Fragaria x ananassa* Duch.), das cv. 'Camarosa' 'Candonga' e 'Ventana'. Como tarefas, destacaram-se a instalação do ensaio em cultivo sem solo, instalando as modalidades em estudo. Efetuou o registo semanal dos parâmetros selecionados, avaliou o efeito de diferentes concentrações de Ca no crescimento vegetativo e nos parâmetros de qualidade do fruto. Concluiu-se que as concentrações mais altas de Ca não influenciaram a firmeza do fruto, aspeto importante no posterior transporte para comercialização. Concluiu-se ainda que foi a cv. 'Candonga' que produziu frutos com maior firmeza, enquanto os frutos da cv. 'Camarosa' *se* destacaram pelo teor de açúcares. As diferenças na qualidade do fruto foram motivadas pelas cultivares e não pelos diferentes níveis de Ca aplicados.

A5. P. Palencia, F. Martinez, E. Ribeiro, M. Pestana, F. Gama**, T. Saavedra**, A. de Varennes, P.J. Correia **(2010)** Relationship between *tipburn* and leaf mineral composition in strawberry. *Scientia Horticulturae* 126: 242-246.

doi:10.1016/j.scienta.2010.07.024

O objetivo do trabalho baseou-se em avaliar as relações entre a incidência de *tipburn* em morangueiro (*Fragaria x ananassa* Duch.) e a aplicação de Ca na solução de rega. Participou no acompanhamento do ensaio em cultivo sem solo, na preparação de soluções nutritivas para fertirrega. Instalou o ensaio de acordo com o desenho experimental estabelecido. Efetuou o registo semanal dos parâmetros, bem como, todas as práticas culturais necessárias incluindo as colheitas semanais. Concluiu-se que este desequilíbrio nutricional parece estar mais relacionado com o crescimento vegetativo e variação sazonal do que com a disponibilidade do Ca no substrato, uma vez que o equilíbrio dos nutrientes K, Ca e Mg nas folhas de algumas cultivares dependem da taxa de transpiração, e esta por sua vez, está associada á temperatura e humidade. A única maneira de evitar a incidência de *tipburn* parece ser com aplicações foliares de Ca.

A6. M. Pestana, F. Gama, T. **Saavedra**, P.J. Correia, S. Dandlen and M.G. Miguel **(2010)** Evaluation of Fe deficiency on strawberry fruit quality. *Ata Horticulturae* 868:423-428.

O objetivo deste trabalho foi o de avaliar os efeitos da deficiência de Fe nas propriedades antioxidantes morango cultivar Selva. Colaborou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos dados. Determinou as atividades antioxidantes em frutos nomeadamente, DPPH (Free Radical Scavenging Activity), TEAC (Trolox Equivalent Antioxidant Capacity, ORAC (Oxygen Radical Absorbance Capacity) e Poder Redutor. Concluiu-se que frutos colhidos com aspeto exterior semelhante apresentam no entanto, um atraso na maturação devido à alteração da qualidade interna (antocianinas e fenóis totais) quando provenientes de plantas sem Fe.

10.3 ARTIGOS PUBLICADOS EM ATAS DE CONGRESSOS NACIONAIS OU INTERNACIONAIS (AC)

AC1. Pestana, M.; Gama, F.; **Saavedra, T**.; Castro Pinto, J.; Abadía, A.; Varennes, A. de; Correia, P.J. **(2012)** A caraterização e correção da deficiência de Fe em plantas de morangueiro: novas abordagens. Atas Portuguesas de Horticultura, 20: 29-34.

Neste trabalho reuniram-se de forma resumida os resultados obtidos em diversos ensaios com plantas de morangueiro (*Fragaria × ananassa* Duch.) destacando-se o estudo dos mecanismos fisiológicos e bioquímicos de controlo da deficiência de Fe e a avaliação de novas alternativas para a correção da clorose férrica.

AC2. T. Saavedra, F. Gama, P.J. Correia and M. Pestana **(2009)** Deficiência de Fe em plantas de morangueiro: efeitos na partição da biomassa e na produção. Atas de Horticultura, 54: 124-125

Estudou-se a deficiência de Fe de plantas de morangueiro (*Fragaria* x *ananassa* Duch) em solução nutritiva, avaliando a biomassa, a produção total e a qualidade dos frutos ao longo do ensaio. Participou no acompanhamento do ensaio em cultivo sem solo, na preparação de soluções nutritivas, efetuou o registo semanal dos dados, fez colheitas semanais e análise nutricional das plantas e frutos e quantificou do teor mineral das plantas sujeitas a diferentes níveis de Fe por absorção atómica-EAA. A clorose férrica antecipou a entrada em floração e em produção do morangueiro, produzindo no primeiro ciclo mais frutos por planta mas mais pequenos. As plantas verdes emitiram mais estolhos mas produziram menos frutos.

AC3. M. Pestana, F. Gama, **T. Saavedra**, S. Dandlen, and M.G. Miguel **(2008)** The effects of Fe deficiency on organic acids, sugars and anthocyanins in strawberry fruits. Livro de Atas do XII Congresso Ibérico sobre a Nutrição Mineral das Plantas. Presente y futuro de la nutrición mineral de las plantas, Eds.: L. Romero, J. Ruiz, L.M. Cervilla, M.M. Wilhelmi, E. Rodriguez, J.J. Rios Universidad de Granada, Espanha, 673-680. (ISBN: 978-84-89780-10-7).

O objetivo do estudo foi o de avaliar o efeito da deficiência de Fe nas características químicas dos frutos de morangueiro colhidos na maturação a partir de plantas desenvolvidas com diferentes concentrações de Fe na solução nutritiva. Colaborou na instalação do ensaio em hidroponia, preparou as soluções nutritivas, efetuou o registo semanal dos parâmetros estabelecidos. Comparativamente aos morangueiros verdes, os cloróticos produziram frutos com peso semelhante, mas com menor intensidade de cor e pobres em características organoléticas.

IV. ANEXOS

AS1

Nutritional partition in Poncirus trifoliata and Ceratonia siliqua grown under different concentrations of iron in nutrient solution

Journal:	*Journal of Plant Nutrition and Soil Science*
Manuscript ID:	jpln.201300005
Wiley - Manuscript type:	Regular Article
Date Submitted by the Author:	03-Jan-2013
Complete List of Authors:	Correia, Pedro; Universidade do Algarve, DCBB, FCT Varennes, Amarilis; CEER, ISA-UTL Gama, Florinda; Universidade do Algarve, DCBB, FCT Saavedra, Teresa; Universidade do Algarve, DCBB, FCT Pestana, Maribela; Universidade do Algarve, DCBB, FCT
Research Area:	Iron, Nutrient deficiency
Manuscript Keyword:	Carob, iron chlorosis, nutrients ratios, citrus, Hydroponics

AS2

International Journal of Food Properties

Taylor & Francis
Taylor & Francis Group

STABILITY AND SENSORY CHARACTERISTICS OF OLIVE OILS DURING STORAGE IN THE PRESENCE OF THYMBRA CAPITATA ESSENTIAL OIL

Journal:	*International Journal of Food Properties*
Manuscript ID:	LJFP-2012-0563
Manuscript Type:	Original Article
Date Submitted by the Author:	13-Oct-2012
Complete List of Authors:	Saavedra, Teresa; Universidade do Algarve, Antunes, Maria; Universidade do Algarve, Dandlen, Susana; Universidade do Algarve, n, Maria; Universidade do Algarve, Martins, Maria; Universidade do Algarve, Figueiredo, Ana; Universidade de Lisboa, Pedro, Luis; Universidade de Lisboa, Barroso, José; Universidade de Lisboa, Miguel, Maria; Universidade do Algarve,
Keywords:	antioxidant, autooxidation, flavor, food stability, food property

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Preview

From: jplantnutrition@gmail.com

To: fpestana@ualg.pt

CC:

Subject: Journal of Plant Nutrition - Manuscript ID LPLA-2011-0076

Body: @@date to be populated upon sending@@

Dear Professor Pestana:

Your manuscript entitled "THE ROOT FERRIC CHELATE REDUCTASE IS REGULATED BY IRON AND COPPER IN STRAWBERRY PLANTS" has been successfully submitted online and is presently being given full consideration for publication in Journal of Plant Nutrition.

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Sincerely,
Journal of Plant Nutrition Editorial Office

Date Sent: 25-Feb-2011

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THE ROOT FERRIC CHELATE REDUCTASE IS REGULATED BY IRON AND COPPER IN STRAWBERRY PLANTS

Journal:	*Journal of Plant Nutrition*
Manuscript ID:	LPLA-2011-0076
Manuscript Type:	Original Articles
Date Submitted by the Author:	25-Feb-2011
Complete List of Authors:	Pestana, Maribela; Universidade do Algarve, FCT Correia, Pedro; Universidade do Algarve, FCT Saavedra, Teresa; Universidade do Algarve, FCT Gama, Florinda; Universidade do Algarve, FCT Dandlen, Susana; Universidade do Algarve, FCT Nolasco, Gustavo; Universidade do Algarve, FCT Varennes, Amarilis; ISA-UTL
Keywords:	Iron < Micronutrients, General Plant Nutrition, Greenhouse Crops

A1

Short running title: The role of Fe and Cu in FC-R

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THE ROOT FERRIC CHELATE REDUCTASE IS REGULATED BY IRON AND COPPER IN STRAWBERRY PLANTS

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ABSTRACT

In the present experiment, we studied the interaction between Cu and Fe in strawberry plants grown in nutrient solutions containing different concentrations of Fe. Plants grown in absence of iron (Fe0) had the characteristic symptoms of Fe deficiency, with smaller chlorotic leaves, less biomass, acidification of the nutrient solution, and roots that were smaller and less ramified, while no symptoms of Fe deficiency were observed in plants grown with Fe. A greater amount of Cu was found in roots of chlorotic plants than in those grown with Fe, while plants grown with 20 µM of Fe (Fe20) in the nutrient solution had a greater amount of Fe compared with plants from the other treatments. Chlorotic plants (Fe0) and plants grown with the greatest level of Fe (Fe20) had a greater root ferric chelate

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reductase (FC-R; EC 1.16.1.17) activity compared with the other treatments with 5 or 10 μM Fe in the nutrient solution. The same pattern was obtained for relative FC-R mRNA concentration and for the sum of Fe and Cu contents in shoots (leaves plus crowns). The DNA obtained from amplification of the FC-R mRNA was cloned and several of the inserts analysed by single strand confirmation polymorphism (SSCP). Although there were different SSCP patterns in the Fe20 treatment, all the inserts that were sequenced were very similar, excluding the hypothesis of more than one FC-R mRNA species being present. The results suggest that Cu as well as Fe is involved in FC-R expression and activity, although the mechanism involved in this regulation is unknown so far. Both small contents of Fe and Cu in plants led to an over-expression of the FC-R gene and enhanced FC-R activity in strawberry roots.

Keywords: copper; ferric chelate reductase (FC-R); FC-R mRNA; iron deficiency; nutrient content; strawberry

1. Introduction

Iron (Fe) can change its oxidation state between (II) and (III) and forms stable octahedral complexes with various ligands, such as Fe-S clusters, that result in different redox potentials in a number of key cellular processes (Palmer and Guerinot 2009). Consequently, Fe is essential in metabolic processes such as photosynthesis, the electron transport chain and chlorophyll biosynthesis (Abadía and Abadía 1993). Iron deficiency also leads to nutrient imbalances in plants, and limits yield and quality (Pestana, de Varennes, and Faria 2003). According to Jeong and Guerinot (2009), understanding plant Fe homeostasis is crucial to improve crop yield and secure a healthy human diet. Recently, several reports (Jeong and Guerinot 2009; Morrissey and Guerinot 2009; Palmer and

Guerinot 2009) summarized the most relevant information on plant Fe acquisition, transport, utilization and homeostasis in plants.

Although Fe is the fourth most abundant element in the earth's crust, in most soils the concentration of ionic iron (Fe^{3+} and Fe^{2+}) in solution is very small, usually less than 10^{-15} M (Marschner and Römheld 1995). Plants have mechanisms that promote the availability of Fe in the rhizosphere and they are separated phylogenetically into two groups: those following Strategy I, and those that adopt Strategy II (Marschner, Römheld, and Kissel 1986). Strategy I is found in dicot and monocot species, with the exception of members of the *Poaceae* (*Gramineae*) family. It includes the induction of three mechanisms localized at the plasma membrane of root cells: i) proton extrusion with acidification of the rhizosphere, ii) a ferric chelate reductase (FC-R) that converts Fe(III)-chelates to Fe(II) and, iii) and a Fe(II) transporter that allows Fe to cross the root plasmalemma (Abadía et al. 2011; Walker and Connolly 2008). Physiological adaptations of Strategy I plants may be associated with morphological root changes such as subapical swelling of roots, formation of new root tips extending from the swollen zones, and formation of root hairs and transfer cells (Landsberg, 1995).

The Ferric Reductase Oxidase (*FRO2*) gene that encodes the inducible FC-R, and the *IRT1* gene, that encodes the high affinity Fe(II) transporter, were first identified in *Arabidopsis thaliana* (Vert, Briat, and Curie 2001). (Connolly et al. 2003) reported a coordinated control of the expression of *FRO2* and *IRT1*, as the first is required to provide the Fe(II) that is the substrate for the *IRT1* product. These authors also stated that post transcriptional regulation of *FRO2* may avoid the accumulation of potentially toxic amounts of Fe, as greater *FRO2* mRNA levels resulted in enhanced FC-R activity only when plants were starved of Fe.

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Large concentrations of other cationic micronutrients (manganese, copper and zinc) may impair Fe nutrition as they compete for ligands both in soils and plants (Wallace, Wallace, and Cha 1992). For example, copper (Cu) can competitively inhibit access of Fe to chelators, thereby decreasing its uptake from soil (Schmidt et al. 1997). Copper also exists in multiple redox states, and acts as co-factor for components of the electron transport chain in mitochondria and chloroplasts (Palmer and Guerinot 2009). Like Fe, Cu also has to be reduced before uptake by its respective transporter (Puig et al. 2007). According to Palmer and Guerinot (2009) *FRO2* expression is not up-regulated by Cu deficiency, but when induced by Fe deficiency, the *FRO2* product is also able to reduce Cu.

A connection between Cu and Fe homeostasis had been suggested. The first observation was obtained in rats, which can overcome anaemia by Cu supplementation (Fox 2003). Interactions between both metals remain unclear in vascular plants but there is a certain similitude between the roles of Fe and Cu in proteins: cytochrome oxidase *versus* diiron oxidase, copper *versu*s haem nitrite reductases, and Cu/Zinc superoxide dismutase *versus* Fe superoxide dismutase (Cohu and Pilon 2007). Depending on metal bioavailability, the use of Cu- versus Fe-containing enzymes to catalyse the same biochemical reaction in plants was reported by Puig et al. (2007).

In the present experiment, we studied the interaction between Cu and Fe in strawberry plants grown in the presence of different concentrations of Fe. The hypothesis tested was that expression and activity of the FC-R activity could be influenced by both metals.

2. Material and methods

Strawberry (*Fragaria x ananassa* Duch. cv. 'Selva') bare root plants (with root length of approximately 18 cm) without leaves were acquired in a nursery. Plants were sterilised by

immersion in a solution with 2.5g fosethyl-aluminium for 2 h and rinsed with running water, before transfer to 20 l containers filled with a nutrient solution containing (in mM) 5.0 $Ca(NO_3)_2$, 1.4 KNO_3, 0.6 K_2SO_4, 1.0 $MgSO_4$, 0.9 NaCl, 0.6 $(NH_4)_2HPO_4$, 3.0 $(NH_4)_2SO_4$, 0.2 $MgCl_2$, and (in µM) 41.8 H_3BO_3, 3.8 $ZnSO_4$, 3.9 $CuSO_4$, 6.9 $MnSO_4$ and 1.0 $(NH_4)_6Mo_7O_{24}$. Iron was added to the solutions as Fe(III)-EDDHA at four different concentrations, 0 (Fe0), 5 (Fe5), 10 (Fe10) and 20 µM Fe (Fe20). The pH of nutritive solutions was monitored twice a week in all containers using a portable pH meter (Hanna Instruments, Germany). Initial pH of the solutions was adjusted to 6.5 ± 0.1.

Plants were grown in a glasshouse for 6 weeks (42 days) under natural photoperiod conditions and temperature ≤ 25 ºC. For each treatment, three containers were used, with six plants per container. The nutrient solutions were constantly aerated.

2.1. Evaluation of iron deficiency

Degree of chlorosis, which is normally used to estimate total chlorophyll (Chl) concentration (Abadía and Abadía 1993), was evaluated using a portable SPAD-502 meter (Minolta, Osaka, Japan). From two weeks after the beginning of the experiment, when leaves had a size that allowed SPAD readings, these were taken twice a week, in each plant, and in at least three of the youngest fully expanded leaves with five readings per leaf.

2.2. Biomass and contents of Fe and Cu

At the end of the experiment (42 days after transplant), at three plants from each treatment were collected. The plant material was separated into roots, crowns and leaves, and the dry weight of each part was determined after drying at 60 ºC until constant weight. The plant material was then ground, ashed at 450 ºC, and digested in 1 M HCl. The concentration of Fe and Cu was determined by atomic absorption spectrophotometry (Pye Unicam,

Cambridge, UK) following standard methods (A.O.A.C. 1990). Iron and Cu contents (µg) were calculated by multiplying the dry weight of each plant part by its corresponding nutrient concentration.

2.3. Activity of root ferric chelate reductase

The activity of root FC-R (EC 1.16.1.17) activity was measured by the formation of the Fe(II)-bathophenantrolinedisulfonate (BPDS) complex from Fe(III)-EDTA (Bienfait et al. 1983). Measurements were performed at the end of the experiment, with at least seven root tips excised with a razor blade from each of three plants per treatment. Each excised root tip (approximately 2 cm, 1.40 ± 0.35 mg fresh mass) was incubated in an Eppendorf tube in the dark with 900 µL of micronutrient-free half Hoagland's nutrient solution, containing 300 µM BPDS, 500 µM Fe(III)-EDTA and 5 mM MES, pH 6.0. Readings were done after centrifugation, one hour after starting the incubation. An extinction coefficient of 22.14 mM cm^{-1} was used. Blank controls without root tips were also used to correct for any unspecific Fe reduction.

Relative FC-R activity was calculated in relation to plants grown with the smallest concentration of Fe (Fe5) for which leaves remained green. This expressed the increment in the activity of this enzyme in Fe-stressed plants compared with Fe-sufficient plants.

2.4. Expression of root ferric chelate reductase

Plant RNA was extracted from 100 mg of roots from three plants from each treatment using the RNeasy Plant Mini Kit (Qiagen). The mRNA was quantified by real time reverse transcriptional polymerase chain reaction (RT-PCR) in an iCycler IQ (Biorad) using two

primers based on the sequence DY667388 FVC0012669_1 available on the database PartiGeneDB (http://www.compsysbio.org/ partigene/cluster.php?cluster=FVC0012669):

FC-RFwd: 5'-TGGTATTAGCAGTGGCAATGTG-3' and

FC-RRev: 5'-CGACGGCAAAGAGGAAGATTC-3'

These will amplify a sequence of 174 bp corresponding to part of the gene for the root FC-R from *Fragaria vesca* L.. The amplifications were done in volumes of 25 µL with 1 µL of RNA template, using the iScript One-Step RT-PCR Kit with SYBR Green (Biorad,) and included 200 nM of each of the FC-R forward and reverse primers. Thermal cycling consisted of 38º for 45 min for reverse transcription, 94º for 2 min for RTase inactivation and factor wells collection, followed by 30 cycles of 92º for 30 sec, 52º for 45 sec and 72º for 45 min, and a final extension step at 72º for 5 min. Each amplification was repeated three times for each sample. Specificity of the amplifications was assessed by melting curve analysis. Relative quantification of FC-R expression was done according to the method of (Pfaffl 2001), using 18S RNA amplification as a normalizing gene. Conditions for 18S RNA amplification were the same as used for FC-R, except for the primers which were:

18SFwd: 5'-GACTACGTCCCTGCCCTTTG-3' and

18SRev: 5'-TGATAAGGTTCAATGGACTTCTTCG -3'.

2.5. Cloning and sequence analysis of FC-R

The RT-PCR products were cloned using the pGEM-T Easy Vector System (Promega Corp, Madison, WI, USA) according to the manufacturer's instructions, and used to transform competent *Escherichia coli* cells.

Several of the white colonies produced were picked and checked by PCR in a reaction mixture containing 1 U of Dream Taq (Fermentas), 1X Buffer taq polymerase, 0.2 mM $MgCL_{2,}$ 0.2 mM of each dNTP and 200 nM of each primer FC-RFwd and FC-RRev; thermal cycling was the same as described above but omitting the reverse transcription step. The

PCR products which consisted of a single band of 174 bp were further characterized by single strand confirmation polymorphism (SSCP). They were mixed with denaturating buffer (95% formamide, 10mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol), heated for 10 min at 100 ºC and chilled on ice for 5 min. The samples were loaded into a 8% non-denaturing polyacrylamide (acrylamide: bisacrylamide, 49:1) gel and the electrophoresis was carried out at 200 V and 4ºC in 1× Tris-borate EDTA buffer during 3.5 h. The gel was silver stained. The band patterns obtained were very similar for the Fe0 treatment, but for the Fe20 treatment they depicted some diversity. Therefore, the inserts corresponding to the colonies originating different patterns from treatment Fe20, as well as three similar patterns from Fe0, were sequenced in both directions using M13 universal primers.

2.6. Statistical analysis

Containers were distributed according to a complete randomized design and each plant was considered a repetition in a total of 18 plants per treatment. The effects of Fe treatments were evaluated by analysis of variance and the means compared using the Duncan Multiple Range Test (DMRT) at $P<0.05$, using the SPSS software. Pierson correlations between some parameters were tested at $P<0.05$.

3. Results

3.1. Development of iron deficiency

Two weeks after transplant, all plants were similar in shape and size, and the average SPAD value in young leaves was 34 ± 1 (Figure1). Twenty six days after the beginning of the experiment, plants grown in absence of iron (Fe0) had significantly smaller SPAD values compared with the other treatments and by day 29 symptoms of iron deficiency became

visible to the naked eye. In these plants SPAD readings decreased until the end of the experiment. In contrast, no symptoms of Fe deficiency were observed in plants grown with Fe and SPAD values ranged from 29 to 34 (Figure 1).

In the absence of Fe, the pH of the nutrient solution decreased from the original 6.5 to about 5.5, while the pH of the nutrient solutions with Fe remained 6.5. For plants grown without Fe, the pH of the nutrient solution was positively correlated with SPAD values in young leaves (R^2=0.95; P<0.05), i.e. as the severity of leaf symptoms increased, so did the acidity of the nutrient solution.

In general, strawberry plants grown without Fe had smaller leaves, and produced less dry matter (Table 1) than plants from the other treatments. They also had different root morphology as these were smaller and less ramified, but with a similar biomass compared with other treatments.

3.2. Iron and copper contents

Plants grown with 20 µM of Fe in the nutrient solution had a greater amount of Fe compared with plants from the other treatments, both in roots, crowns and leaves (Figure 2). The amount of Cu in crowns increased with the supply of Fe in the nutrient solution. In contrast, a greater amount of Cu was found in roots of chlorotic plants than in those grown with Fe (Figure 2).

3.3. Expression and activity of ferric chelate reductase

Chlorotic plants and plants grown with the greatest level of Fe had a greater FC-R activity compared with the other treatments (Figure 3). The same pattern was obtained for relative mRNA concentration, and for the sum of Fe and Cu contents (Figure 4).

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Although there were different SSCP patterns in the Fe0 and Fe20 treatments (Figure 5), all the inserts that were sequenced were very similar, differing only between 1 to 6 nucleotides (0.6-3.5%) from the Genbank reference sequence DY667388. This excluded the hypothesis of more than one mRNA species being detected by RT-PCR as the small differences could be attributed to the usual error rates of the reverse transcriptase and *Taq* DNA polymerase.

4. Discussion

Results from this study show the response mechanism of strawberry plants to Fe deficiency, which is in accordance to those previously described for Strategy I plants, and reports a link between Fe and Cu levels and root FC-R activity.

Twenty-six days after the beginning of the experiment and until the end, strawberry plants grown without Fe (Fe0) in nutrient solution developed the typical symptoms of iron chlorosis, which became apparent as an interveinal chlorosis and occurred primarily in young leaves. After this date, the degree of chlorosis was markedly increased with time, as leaf chlorophyll concentration decreased, and emerging new leaves were completely yellow.

The roots of plants grown without Fe were smaller and less ramified (but with a similar biomass) than those from the other treatments. These changes were similar to those reported in other species such as in citrus (Pestana et al. 2005) and carob rootstocks (Correia, Pestana, and Martins-Loução 2003) and sugar beet (Landsberg 1995) when grown with small levels of iron or in the absence of this element. The plants of the other treatments grown with Fe (Fe5, Fe10 and Fe 20) remained green until the end of the experiment, and had no morphological differences in leaves and roots.

The morphological changes were associated with acidification of the nutrient solution and with enhanced FC-R activity, again as described for Strategy I plants. The increment of root proton extrusion is mediated by H^+-ATPases located in root plasma membranes (Susín et al. 1994) and seemed to be regulated by a shoot signal, as the degree of chlorosis in leaves was related with the decrease in the pH of the nutrient solution. There is probably a critical level of leaf chlorophyll content that triggers the process of medium acidification, as previously reported in subterranean clover (Wei, Loeppert, and Ocumpaugh 1998).

The root FC-R activity measured in strawberry roots was within the ranges proposed for other species (Pestana et al. 2005; Zouari, Abadía, and Abadía 2001). Increases in FC-R activities are frequently observed in dicots cultivated with low levels of Fe, and this has been assumed to arise from an inducible plasma membrane-bound FC-R enzyme(s) (Zheng et al. 2003). Zheng et al. (2003) suggested that a temporal autonomy between proton extrusion and FC-R activity is present, representing an effective response of Strategy I plants, both from an energetic and an ecological point of view. The different pattern of response supported by our results (association of proton extrusion and FC-R activity) could be explained by the fact that chlorotic plants were kept in a nutrient solution without Fe at all times, with no possibility of Fe trafficking (as a signal) from roots to leaves.

Compared to the other treatments, Cu content in roots of chlorotic plants grown without Fe increased 3-fold, indicating that Cu may be taken-up instead of Fe, again in agreement with the results obtained in other species where the FC-R activity induced by Fe deficiency was able to reduce Cu (Palmer and Guerinot 2009). In conclusion, all the results discussed so far point to strawberry being a typical Strategy I plant species.

Unexpectedly, strawberry plants grown with the greatest Fe concentration (Fe20) also presented an increase in root FC-R activity, although without any symptoms of either Fe

deficiency or toxicity. This enhanced activity led to an increase in Fe uptake, and as a consequence the total amounts of Fe in plants increased, especially in roots (5-fold increase).

The enhanced activity of FC-R was related to over-expression of its correspondent mRNA demonstrating that gene regulation was involved in this response. What could have caused this increase in the expression of the *FRO* gene? We evaluated the concentration of all cationic micronutrients and found that the sum of Fe plus Cu contents was related with the FC-R activity and levels of FC-R mRNA (no similar correspondence was found with manganese or zinc, data not shown). This pointed to an involvement of Cu in the regulation of the *FRC* gene that has not been reported before. The next obvious question was: is it the same gene that is being over-expressed or the mRNA detected in plants grown with a great level of Fe corresponds to a family of FC-R proteins? To answer this question, the DNA obtained from the amplification of the mRNA extracted from these plants and from chlorotic plants grown in the absence of Fe was cloned and sequenced. Initially, it seemed that a family of similar mRNAs was involved as there were several band patterns present when the inserts were analysed by SSCP. However, when these were sequenced it became clear that only one mRNA species was involved (at least in the 174 bp that were amplified).

The final question was: why is Cu involved in FC-R expression and activity? As stated in the introduction, there seems to be a connection between Cu and Fe homeostasis. These metals are both present in proteins that perform similar roles in plant metabolism (Cohu and Pilon 2007), and there is some evidence that in some cases Cu- versus Fe-containing enzymes may catalyse the same biochemical reactions (Puig et al. 2007). Therefore it makes sense that the FC-R is controlled by both metals. The mechanism involved in this regulation is unknown so far.

Acknowledgements

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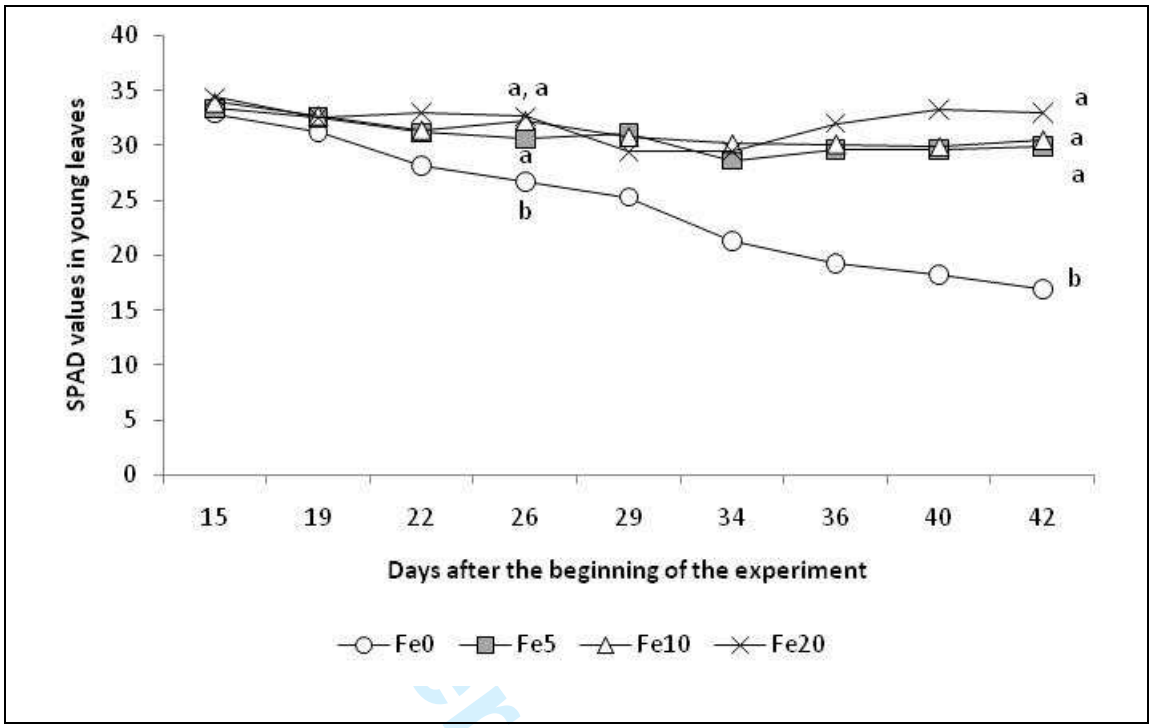

Figure 1 - Changes in SPAD values with time in young leaves of strawberry plants as affected Fe level in nutrient solution. Statistical analysis is presented only at two dates (26 and 42 days after the beginning of the experiment). In each date, means with the same letter in each column were not significantly different ($P < 0.05$), using the Duncan multiple range test.

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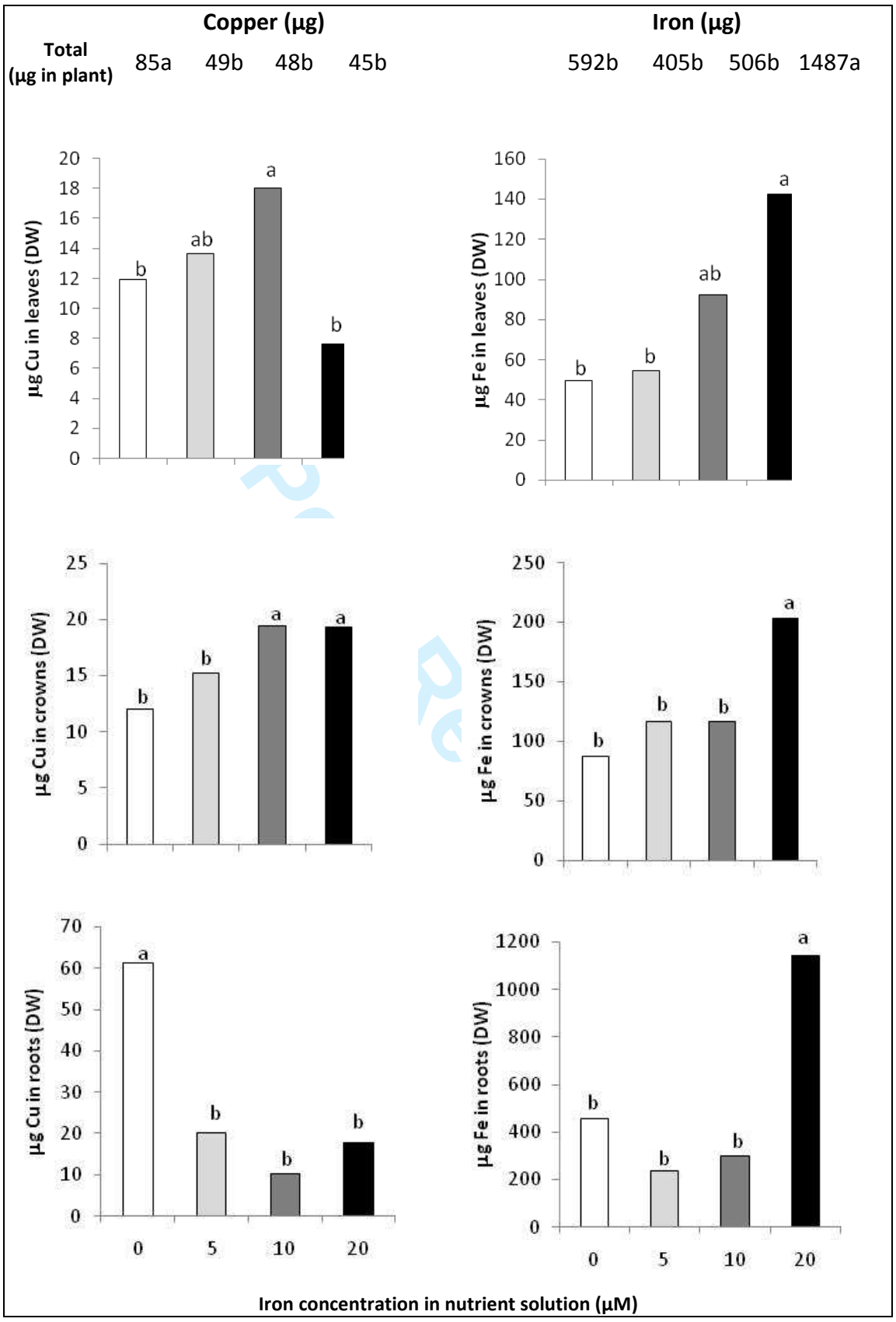
Copper (µg)
Iron (µg)
Total (µg in plant)
85a 49b 48b 45b
592b 405b 506b 1487a
µg Cu in leaves (DW)
µg Fe in leaves (DW)
µg Cu in crowns (DW)
µg Fe in crowns (DW)
µg Cu in roots (DW)
µg Fe in roots (DW)
Iron concentration in nutrient solution (µM)

A1

Figure 2 – Contents (µg) of Fe and Cu in roots, crowns and leaves of strawberry plants grown with different Fe concentrations in nutrient solution, at the end of the experiment (42 days after the beginning of the experiment). Total Fe and Cu contents are also presented. DW – dry weight. Columns with the same letter are not significantly different ($P < 0.05$), using Duncan multiple range test.

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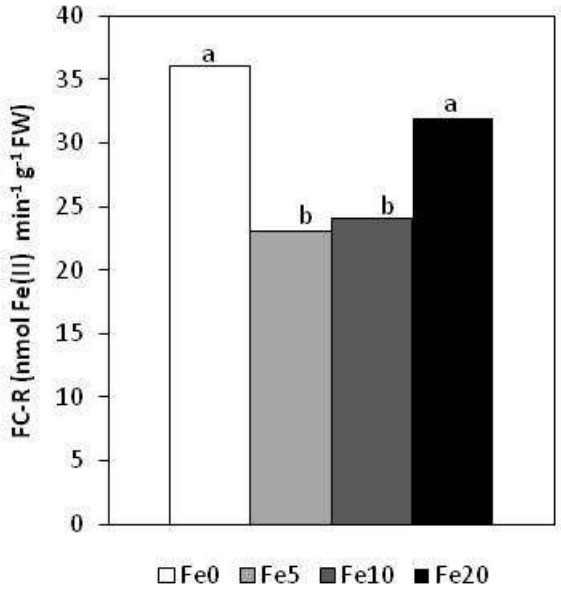

Figure 3 - Root tips ferric chelate-reductase (FC-R) activities (nmol Fe reduced min^{-1} g^{-1} fresh weight) of strawberry plants grown with different Fe concentrations in nutrient solution, at the end of the experiment Columns with the same letter are not significantly different ($P < 0.05$), using Duncan multiple range test.

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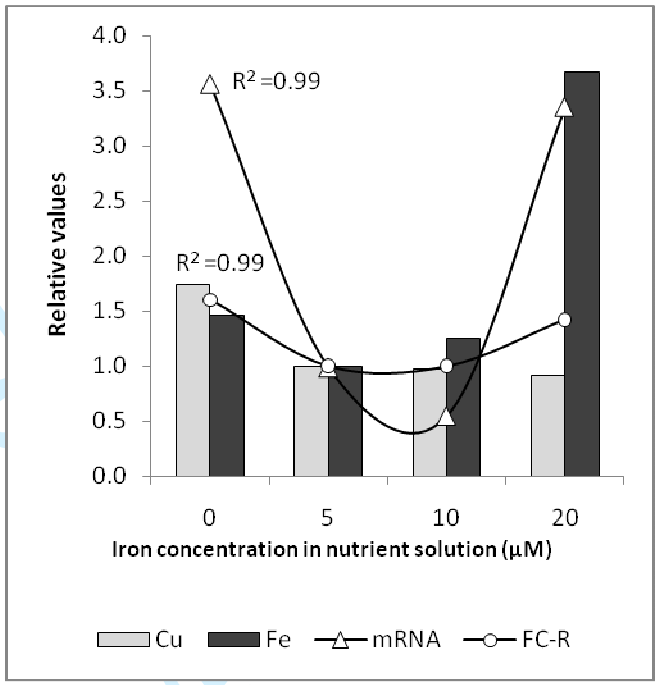

Figure 4 – Relative mRNA expression and relative ferric chelate-reductase (FC-R) activity of root tips of strawberry plants grown with different Fe concentrations in nutrient solution at the end of the experiment (42 days after the beginning of the experiment). Columns with the same letter are not significantly different ($P < 0.05$), using Duncan multiple range test.

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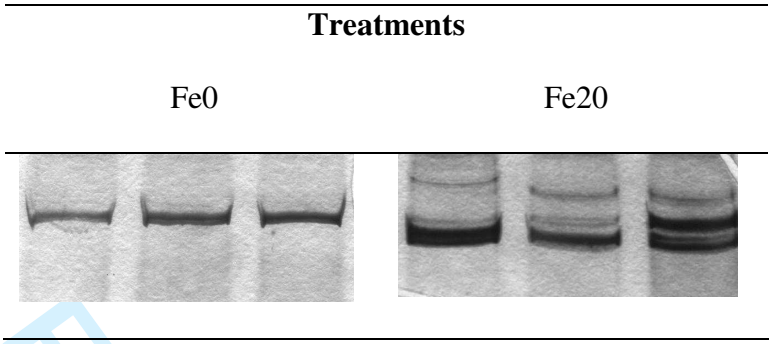

Figure 5 - SSCP patterns obtained from bacterial clones positive for the FR-C fragment from strawberry plants grown without Fe (Fe0) and with 20 µM Fe in the nutrient solution (Fe20).

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Table 1. Biomass accumulation in leaves, crowns and roots of strawberry plants grown with different levels of Fe in the nutrient solution. The root: shoot (crowns plus leaves) values are also presented.

Treatments Fe (µM)	**Dry weight (g)**			
	Leaves	Crowns	Roots	Root: Shoot
0	0.93 b	0.70 a	0.87 a	0.53 a
5	1.20 ab	0.65 a	0.36 a	0.20 b
10	1.47 ab	0.77 a	0.50 a	0.22 b
20	1.80 a	1.10 a	0.87 a	0.30 b

Columns with the same letter are not significantly different at $P<0.05$, using the Duncan multiple range test.

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Short communication

Development and recovery of iron deficiency by iron resupply to roots or leaves of strawberry plants

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ABSTRACT

Bare-root transplants of strawberry (*Fragaria ananassa* Duch. cv. 'Selva') were transferred to nutrient solutions with or without iron (Fe). After six weeks of growth, plants grown in solution lacking Fe were chlorotic and showed morphological changes in roots typical of Fe deficiency. Subsequently, four treatments were applied for nine days: plants grown in continued absence of Fe (Fe0); plants grown in continued presence of 10 μM Fe (Fe10); foliar application of ferrous sulphate every two days to chlorotic plants (Fe-leaves); and growth of chlorotic plants in solution with ferrous sulphate (Fe-solution). After six days, the chlorophyll (Chl) content in leaves of Fe-solution plants was similar to that in Fe10 plants. Under the Fe-leaves treatment, a slight regreening of new leaves was observed only by the end of the experiment. After nine days, ferric chelate reductase (FC-R) activity was unchanged in Fe10 but increased in Fe0 plants. The FC-R activity of Fe-solution plants was similar to the initial value for chlorotic plants, whereas it was reduced drastically under the Fe-leaves treatment. The Fe concentration in leaves of Fe0 and Fe10 was similar, whereas the Fe-solution and Fe-leaves treatments enhanced leaf Fe concentration. In contrast to the Fe-solution treatment, foliar application of Fe did not increase the Fe concentration in roots. Under our experimental conditions, FC-R activity in strawberry appeared to be deactivated rapidly by pulses of Fe applied by foliar sprays. Deactivation was slower if Fe was applied directly to roots, which suggested that the plants had greater opportunity to take Fe.

1. Introduction

Iron deficiency (iron chlorosis) is an important nutritional disorder in fruit trees that results from impaired acquisition and use of the metal by plants, rather than from a low level of Fe in soils. The most evident effect of Fe deficiency is a decreased content of photosynthetic pigments, which results in the relative enrichment of carotenoids over chlorophylls (Chl) and leads to the yellow colour that is characteristic of chlorotic leaves [1].

Plants employ mechanisms that promote Fe availability in the rhizosphere and plant. Dicot and monocot species, with the exception of members of the family Poaceae, have developed a strategy (Strategy I) that involves the induction of a ferric chelate reductase (FC-R; EC 1.16.1.17) in roots that converts Fe(III) to Fe(II), which can then be taken up by an Fe(II) transporter [2,3]. Excretion of organic acids from roots to the rhizosphere can improve Fe availability further, and accumulation of these compounds in Fe-deficient plants can also stimulate long-distance transport of the metal [4].

Large concentrations of calcium carbonate, as in calcareous soils, result in high levels of bicarbonate ions, which are the main cause of Fe deficiency. Countries in southern Europe, such as Portugal, Spain, Italy, and Greece, have large areas of calcareous soils that contain established orchards. In these orchards, Fe chlorosis is a major factor that limits growth, yield, and profitability [5–7]. Crops that are commonly affected by Fe deficiency when grown in calcareous soils include apple, blueberry, cherry, citrus, corn, grape, turf and pasture grasses, peach, pear, plum, quince, sorghum, soybean, and strawberry [5–10]. When grown on calcareous soils, strawberry production may be affected seriously by induced Fe deficiency. Strawberry shows wide genotypic variation in tolerance

Abbreviations: BPDS, Fe(II)–bathophenantrolinedisulfonate; Chl, chlorophyll; DW, dry weight; EC, electrical conductivity; EDDHA, ethylenediamine-N-N'bis(o-hydroxyphenylacetic) acid; EDTA, ethylenediamine-tetraacetic acid; FC-R, ferric chelate reductase; FW, fresh weight; MES, 2-(N-morpholino)ethanesulfonic acid; SPAD, soil and plant analyser development.

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to Fe deficiency, and Fe correction is necessary in susceptible cultivars. Iron is applied frequently by fertigation or via foliar sprays. Use of Fe sulphate may be a cheap and environmentally friendly alternative to the use of Fe chelates [7,11,12]. Application of Fe directly to leaves can bypass the inhibitory effects of soil bicarbonate on Fe uptake and transport to the shoot [13,14].

The resupply of Fe to chlorotic plants can induce metabolic changes within a few days or weeks, depending on the parameter and plant material. For example, Fe resupply leads to increases in Chl concentration and the rate of photosynthesis [15,16], and decreases in the concentration of organic acids [4,17]. In addition, the carboxylate concentration in the xylem sap and leaf apoplast is reduced in sugar beet [18] and fruit trees [19]. At root level, deactivation of some Fe acquisition mechanisms, including ferric reductase oxidase (FRO) and iron-regulated transporter (IRT) has been reported [1,4,20,21].

A recent review [1] stated the need to investigate the responses of Fe-deficient plants upon the resupply of Fe, which might provide crucial information for optimization of Fe-fertilization strategies. The aims of this investigation were: (1) to characterize the changes induced by Fe depletion on the Chl content, root FC-R activity, and mineral composition of roots and leaves and (2) to compare plant responses to two different methods of resupplying Fe, foliar and root fertilizer application. Finally, we discuss the agronomical consequences of Fe resupply from fertilizer.

2. Results

2.1. Effects of iron depletion on strawberry plants

Iron deficiency was related to the small concentration of Chl in the leaves of strawberry plants (Fig. 1). After 36 days, plants grown

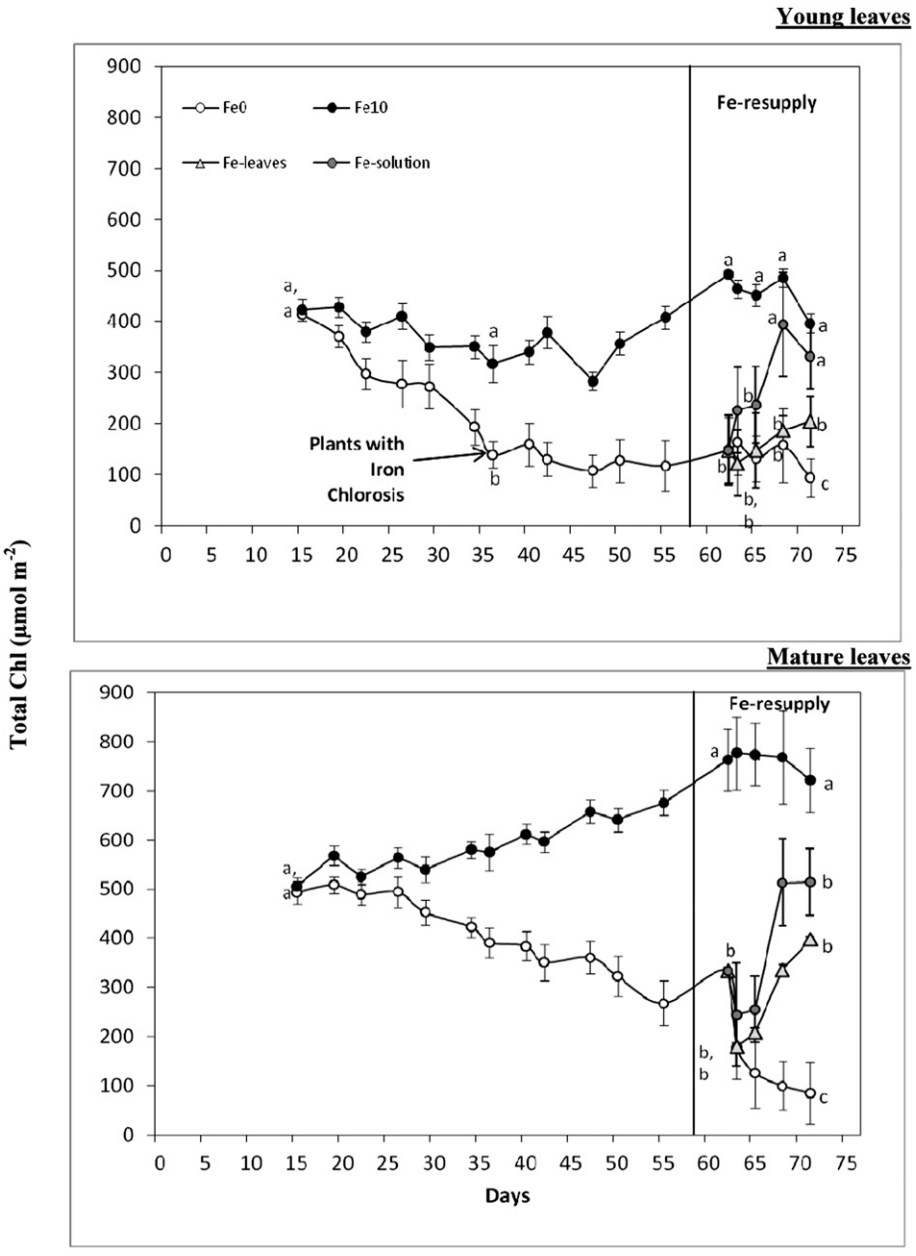

Fig. 1. Leaf chlorophyll (Chl) content in the youngest and mature leaves during the experimental period. For each time point, values (means ± standard deviation) with the same letter were not significantly different (Duncan's test, $P < 0.05$).

in the absence of Fe (Fe0) showed the first symptoms of Fe deficiency in the youngest leaves, which became chlorotic (139 ± 24 μmol Chl m^{-2}), whereas control plants (grown in 10 μM Fe; Fe10) remained green throughout the experimental period and had a Chl content of approximately 395 ± 38 μmol m^{-2}. Leaf fresh and dry weights (Table 1) were less in Fe0 plants than in Fe10 plants.

The root system of Fe0 plants was smaller but more ramified, with lower fresh and dry weights (Table 1), compared with Fe10 plants. Immediately before Fe was resupplied, the pH of the nutrient solution decreased from 6.0 to approximately 4.6 for plants grown in the absence of Fe, whereas the pH of the nutrient solution that contained Fe remained neutral (pH = 6.5). FC-R activity (Fig. 2) was approximately 2.5-fold higher in Fe0 plants than in Fe10 plants (20 ± 2 nmol Fe(II) min^{-1} g^{-1} FW and 8 ± 1 nmol Fe(II) min^{-1} g^{-1} FW respectively).

Iron deficiency also induced changes in the mineral composition of strawberry leaves and roots (Table 1). The leaves of Fe0 plants contained a greater concentration of Zn than those of Fe10 plants. The concentrations of Cu, Mn, and Zn were significantly larger also in the roots of Fe0 plants than in Fe10 plants. The concentration of Fe was least in the leaves and roots of Fe0 plants.

2.2. Effects of Fe resupply to chlorotic strawberry plants

The resupply of Fe to chlorotic plants caused the Chl content in both young and mature leaves to increase (Fig. 1). When Fe was added to the nutrient solution (Fe-solution), leaf regreening was evident after six days. However, in plants whose leaves were sprayed with ferrous sulphate (Fe-leaves), regreening was only partial and the Chl content in mature leaves never reached the values observed in the control Fe10 plants.

At the end of the Fe resupply experiment, FC-R activity remained similar to the initial level in Fe10 plants but had increased even further in the root tips of Fe0 plants (Fig. 2). The FC-R activity in the roots of Fe-solution plants was similar to that observed in Fe0 plants before Fe was resupplied, whereas FC-R activity was reduced drastically when Fe was applied to leaves (Fe-leaves). The Fe concentration in leaves that were sprayed with Fe was greater than that in Fe0 or Fe10 plants. Addition of Fe to the nutrient solution resulted in larger concentrations of Fe in roots than did foliar application of Fe. In the latter treatment, the Fe concentration in roots was similar to that of Fe0 plants (Table 1).

Table 1
Mineral composition in Fe-sufficient (Fe10) and Fe-deficient (Fe0) plants and in plants in which Fe was resupplied by spraying of leaves (Fe-leaves) or in nutrient solution (Fe-solution) as Fe(II)–sulphate. Macronutrients (N, P, K, Mg, and Ca) are in g kg^{-1} dry weight (DW) and micronutrients (Cu, Zn, Mn, and Fe) are in mg kg^{-1} DW.

	Fe-deficient	Fe-sufficient	Fe-resupplied	
	Fe0	Fe10	Fe-solution	Fe-leaves
Leaves				
N	22.9 ± 1.9 ns	24.0 ± 1.0 ns	23.0 ± 0.4 ns	24.8 ± 1.6 ns
P	6.5 ± 1.3 ns	5.8 ± 0.3 ns	9.9 ± 0.7 ns	4.8 ± 0.6 ns
K	37.3 ± 5.2 b	32.8 ± 3.4 b	47.6 ± 5.5 a	40.9 ± 5.5 ab
Mg	4.1 ± 0.6 ab	3.5 ± 0.5 b	4.8 ± 0.3a	4.2 ± 0.3 ab
Ca	13.7 ± 2.1 ns	15.5 ± 0.7 ns	14.9 ± 0.4 ns	12.8 ± 0.7 ns
Cu	20 ± 4 ns	12 ± 2 ns	17 ± 2 ns	13 ± 2 ns
Zn	37 ± 8 a	24 ± 2 b	24 ± 11 b	26 ± 4 ab
Mn	370 ± 74 ns	262 ± 18 ns	265 ± 50 ns	255 ± 58 ns
Fe	59 ± 20 d	84 ± 13 c	173 ± 5 b	275 ± 14 a
FW	8 ± 0.6 b	22 ± 1.5 a	8 ± 2.0 b	7 ± 1.0 b
DW	1 ± 0.1 b	4 ± 0.3 a	2 ± 0.7 b	1 ± 0.5 b
Roots				
N	23.2 ± 1.3 ns	24.3 ± 3.6 ns	19.7 ± 2.6 ns	20.5 ± 4.2 ns
P	5.7 ± 0.8 b	7.0 ± 1.0 b	14.4 ± 2.3 a	6.4 ± 2.3 b
K	17.6 ± 1.3 a	17.8 ± 2.7 a	2.7 ± 0.4 b	14.8 ± 1.0 a
Mg	3.2 ± 0.2 b	4.1 ± 0.6 a	1.1 ± 0.1 c	2.5 ± 0.5 b
Ca	10.8 ± 1.2 a	10.2 ± 1.6 a	5.7 ± 1.1 b	11.7 ± 0.6 b
Cu	126 ± 38 a	30 ± 15 b	31 ± 1.0 b	75 ± 25 ab
Zn	439 ± 113 a	201 ± 36 b	117 ± 42 b	282 ± 89 ab
Mn	685 ± 132 a	377 ± 48 ab	137 ± 56 b	411 ± 198 ab
Fe	374 ± 46 c	593 ± 81 b	1658 ± 347 a	395 ± 73 c
FW	8 ± 0.9 b	12 ± 1.0 a	9 ± 0.7 b	9 ± 1 b
DW	0.9 ± 0.2 b	1.4 ± 0.1 a	0.9 ± 0.1 b	0.7 ± 0.2 b

Data are means ± standard error (SE) of at least three replicates. FW – fresh weight expressed in g; ns – not significant. For each row, values with the same letter were not significantly different (Duncan's test, $P < 0.05$).

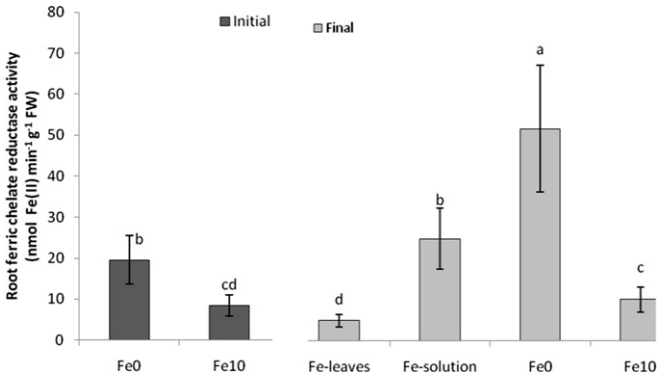

Fig. 2. Root ferric chelate reductase activities at the beginning and end of the experiment, in strawberry plants grown in the absence of Fe (Fe0) or the presence of Fe (Fe10), and in chlorotic plants with Fe added to the nutrient solution (Fe-solution) or applied to leaves (Fe-leaves). For each column, values (means ± standard deviation) with the same letter were not significantly different (Duncan's test, $P < 0.05$).

3. Discussion

Iron fertilizer application improves yield and fruit quality in several crops and is a standard practice in regions of fruit production [5]. Recovery of chlorotic strawberry plants was possible by addition of Fe(II)-sulphate to roots or leaves. The Fe concentration in strawberry leaves decreased markedly under Fe deficiency, but recovered rapidly in response to the application of Fe, either to the leaves or the nutrient solution. However, when Fe was supplied to leaves, regreening was only partial, and the Chl content in new leaves was less than those from Fe10 plants. Partial regreening after foliar spraying of Fe has been observed previously in orange and peach trees [6,10,22]. To be effective, the Fe that is applied exogenously to leaves must enter leaf cell protoplasts and be integrated into plant metabolism [14]. The foliar treatment might have been more effective if a surfactant and an adjuvant had been used, but the interactions between Fe salts and these products remain uncertain [10], and thus they were omitted in the present experiment.

Total leaf regreening was only observed when Fe was added to the nutrient solution. However, the response of strawberry plants to Fe deficiency or Fe resupply seems to be coordinated by shoots and not only by the availability of Fe in the nutrient solution. This shoot-to-root coordination was described first in pea mutants [2]. In the present study, the range of FC-R activities in roots was similar to those reported for other species [9,23–26]. The Fe0 plants showed chlorotic leaves and enhanced root FC-R activity (Fig. 2), whereas control (Fe10) plants had smaller FC-R activity but remained green until the end of the experiment and showed no morphological or physiological changes. The latter demonstrated that the concentration of Fe in the nutrient solution (10 μM) was sufficient for strawberry plants. The root FC-R activity seemed to be deactivated following application of the Fe spray and an increase in leaf Fe content was observed, although leaves only became partially green (Table 1). When Fe was added to the nutrient solution, a large

increase in root Fe concentration was observed (Table 1) and FC-R activity was decreased but not deactivated completely.

The agronomical implications of the deactivation of response mechanisms by foliar sprays are important because in field crops micronutrients are applied mostly to the canopy (leaves and shoots). In addition, an understanding of these mechanisms might help the development of approaches to sustain the regreening effects for a longer period. In fact, it was proposed recently that, in response to canopy sprays, a large consumption of energy can be expected due to the 'on–off' regulation of FC-R [1]. Furthermore, reciprocal grafting experiments with the wild type and a pea mutant that showed elevated FC-R activity indicated that FC-R activity was probably controlled from shoots [2,27]. A decrease in the expression of *NtFRO1* and *NtIRT1* mRNA was observed upon foliar Fe resupply to tobacco plants, which further stressed the importance of shoot signalling in the downregulation of FC-R [20].

The nature of the signal in Fe-deficient plants has not been clarified, although plant hormones, nitrogen monoxide, Fe-binding compounds, and even Fe itself have all been suggested as possible signal molecules for the downregulation of Fe deficiency responses in roots [1]. When Fe was added to the nutrient solution, total recovery from the symptoms of Fe deficiency and an increase in Chl synthesis was observed, which indicated a dependence on the fresh supply of Fe via the xylem; however, a slow deactivation of root FC-R was also detected. The difference in the time course of deactivation of FC-R activity between plants exposed to Fe in the solution and sprayed plants might be related to the difference between the continuous availability of Fe for root uptake in the solution and the pulses of Fe applied by foliar sprays.

In conclusion, under our experimental conditions, FC-R activity in strawberry appears to be deactivated rapidly by pulses of Fe applied by foliar sprays. In contrast, this deactivation mechanism is slower when Fe is applied directly to roots, which suggests a greater opportunity for plants to take up a greater amount of Fe from the rhizosphere.

4. Methods

4.1. Plant material

Bare-root plants (with root length of approximately 18 cm) of strawberry (*Fragaria ananassa* Duch. cv. 'Selva') without leaves were sterilised by immersion in a solution that contained 2.5 g of fosetyl–aluminium for 2 h and then washed thoroughly in running water. Twenty-four plants were transferred to two 20-L polyethylene vessels filled with Hoagland's nutrient solution, which contained: 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1.0 mM KH_2PO_4, 2.0 mM $MgSO_4$, 46.0 μM H_3BO_3, 0.8 μM $ZnSO_4$, 0.4 μM $CuSO_4$, 9.0 μM $MnCl_2$, and 0.02 μM MoO_3. Half of the plants were grown in the presence of 10 μM Fe (as Fe(III)-EDDHA; Fe10) and half in the absence of Fe (Fe0). The initial pH of the nutrient solution was 6.0 ± 0.2 and the electrical conductivity (EC) was 2.2 ± 0.1 dS m^{-1}. The aerated nutrient solution was replaced when the EC dropped to 1.7 dS m^{-1}. The experiments were performed in a glasshouse under natural photoperiod conditions and a temperature ≤25 °C.

After 58 days, Fe10 plants remained green (492 ± 38 μmol Chl m^{-2}) but Fe0 plants were chlorotic (148 ± 24 μmol Chl m^{-2}). At this time point, plants were transferred individually to 1-L glass jars that contained nutrient solution. Four Fe10 plants were grown in the continued presence of 10 μM Fe and four Fe0 plants were grown in the continued absence of Fe (positive and negative controls, respectively). Iron was resupplied to six Fe0 plants by two distinct treatments: (i) foliar spray (Fe-leaves), i.e. plants grown in nutrient solution without Fe were sprayed three times every two days with a solution of 1.8 mM Fe as ferrous sulphate (pH = 5.34 ± 0.01; EC = 0.36 ± 0.00 dS m^{-1}); (ii) nutrient solution (Fe-solution), i.e. plants were transferred to nutrient solution that contained 0.75 mM Fe as ferrous sulphate. In the Fe-leaves treatment, all leaves (approximately five) were sprayed on both the abaxial and adaxial surfaces; a total volume of 83 mL (without a wetting agent or surfactant) was applied to each plant over the course of the three sprays. In conclusion, four treatments were conducted, each with at least three plants (replicates): (1) plants always grown without Fe (Fe0); (2) plants always grown with Fe (Fe10); (3) chlorotic plants sprayed with Fe (Fe-leaves); (4) chlorotic plants transferred to a solution that contained Fe (Fe-solution). For all in the four treatments, plants were grown for nine days in the same glasshouse.

4.2. Leaf chlorophyll determination

New leaves appeared approximately 15 days after the beginning of the experiment and from then on the total Chl concentration was estimated nondestructively in mature and the youngest fully expanded apical leaves using a portable SPAD-502 m (Minolta, Osaka, Japan). Five readings per leaf were recorded for at least three leaves per plant. SPAD readings were converted to total Chl using the equation:

$$Y = 0.4453x^2 - 1.1114x + 32.562 \ (r^2 = 0.98;\ n = 31;\ P < 0.001)$$

where Y is the Chl content (μmol m^{-1}) and x is the SPAD reading [9]. This calibration curve was established by analysing leaf disks that showed different degrees of Fe deficiency with the SPAD-502, extracting the pigments from the same leaf area with 100% acetone in the presence of Na ascorbate [28], and measuring Chl spectrophotometrically according to [29].

4.3. Ferric chelate reductase activity of strawberry root tips

The activity of root FC-R (EC 1.16.1.17) was evaluated in plants both immediately before the four treatments were imposed and at the end of the experiment. The activity of FC-R was measured by formation of the Fe(II)–bathophenantrolinedisulfonate (BPDS) complex from Fe(III)–EDTA [30]. A preliminary test was performed to visualize the location of FC-R activity. Given that active enzyme could be detected clearly by the rose colouration of the root tips (Fig. 3), it was possible to use the following methodology. At least seven root tips were excised with a razor blade from plants from each treatment, and at least 15 measurements of FC-R activity per treatment were recorded. Each excised root tip (approximately 2 cm; 1.40 ± 0.35 mg FW) was incubated in an Eppendorf tube in the dark with 900 μl of micronutrient-free half-strength Hoagland's nutrient solution, which contained 300 μM BPDS, 500 μM Fe(III)–EDTA, and 5 mM MES buffer, pH 6.0. The activity of FC-R was recorded after centrifugation of the samples at 535 nm, 1 h after the start of incubation, using a spectrophotometer (Cadas 100 UV–VIS Photometer; Dr. Lange, Düsseldorf, Germany). Fe(II)-BPDS was quantified using a molar extinction coefficient of 22.14 mM cm^{-1}. Following each assay, the roots were dried gently with blotting paper and the FW was determined. All values for FC-R activity were calculated on a FW basis. Blank controls without root tips were also used to correct for any nonspecific photoreduction.

4.4. Mineral composition analysis

At the end of the experiment, plants from each treatment were harvested and separated into roots and shoots (leaves and petioles). The plant material was washed first with tap water, followed by

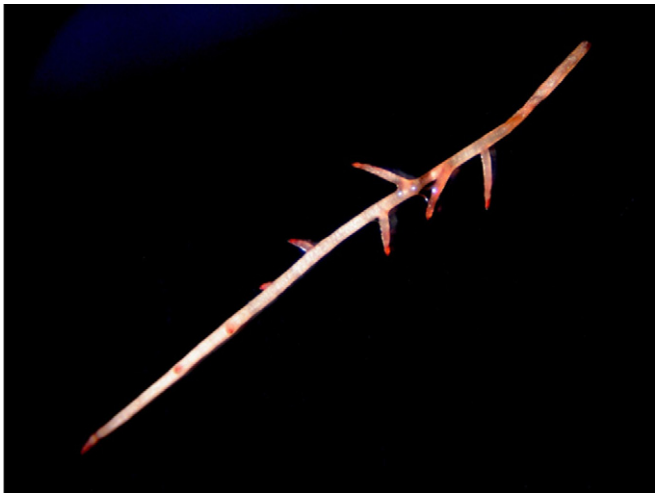

Fig. 3. Rose colouration of root tips indicates localization of FC-R activity.

deionized water that contained a nonionic detergent, and then with 0.01 M HCl. The plant material was rinsed three times with deionized water, dried at 60 °C to a constant weight, and then ground to a powder. The mineral composition was determined as described previously [31]. The N concentration was determined by the Kjeldahl method. Subsamples were dry-ashed at 450 °C and digested in HNO_3 and HCl following the A.O.A.C. procedure [32]. The P concentration was determined spectrophotometrically, whereas those of K, Ca, Mg, Mn, Zn, Fe, and Cu were determined by atomic absorption spectrophotometry (Pye Unicam, Cambridge, UK).

4.5. Statistical analyses

The effects of treatments were evaluated by the general linear model (GLM) and the means compared using Duncan's multiple range test at $P < 0.05$. Statistical analyses were carried out with STATISTICA 10 software (StatSoft Inc., Tulsa, OK, USA).

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Short communication

The root ferric-chelate reductase of *Ceratonia siliqua* (L.) and *Poncirus trifoliata* (L.) Raf. responds differently to a low level of iron

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ABSTRACT

Iron (Fe) deficiency is a common nutritional disorder in several crops grown in calcareous soils, but some species are well adapted to these conditions. A hydroponic experiment was conducted to compare the response of a calcicole species *Ceratonia siliqua* L. (carob) and of *Poncirus trifoliata* (L.) Raf., a citrus rootstock very sensitive to Fe deficiency. Rootstocks from both species were grown in nutrient solutions without Fe (0 μM Fe), with 1 μM Fe, and with 10 μM Fe (carob) or 40 μM Fe (*P. trifoliata*). A low level of Fe or its absence in the nutrient solution led to a significant decrease in *P. trifoliata* vegetative growth and in SPAD readings. The root activity of ferric-chelate reductase (FC-R), a key enzyme in Fe uptake, was low in the absence or with high levels of Fe. Its highest values were in roots exposed to a low level of Fe as described in several sensitive species. In contrast, the activity of FC-R was very high in carob in the absence of Fe and was decreased sharply even when only a low level of Fe was present in the nutrient solution. Plant growth and SPAD readings in the leaves of carob were similar in all treatments. Carob seems to maintain a large activity of root FC-R that may ensure enough Fe to satisfy plant demand. The fact that it presents a slow growing pattern may also contribute to the tolerance of this species to low levels of external Fe.

1. Introduction

Iron (Fe) deficiency is one of the major abiotic stresses of fruit trees in the Mediterranean area of southern Europe. The most important cause of this nutritional deficiency is the low availability of Fe to plants grown in calcareous soils (rich in lime) common in this semi-arid area. In citrus, the tolerance to Fe chlorosis is determined by the rootstock and among these *Poncirus trifoliata* (L.) Raf. is very susceptible to this deficiency (Llosá et al., 2009). The carob tree (*Ceratonia siliqua* L.) is an evergreen species present in the entire Mediterranean basin that plays an important role in the economy of several countries due to the high biotechnological value of the seeds. This crop shares the same edaphoclimatic environment as *Citrus* in southern Portugal. Under these conditions, Fe availability is similar but these two crops behave differently suggesting two different strategies to face this abiotic stress. A comparative study conducted under controlled conditions may reveal those strategies. Carob propagation in commercial orchards is achieved by grafting 2–4-year-old seedlings rootstocks. The rootstocks are obtained from seeds of female plants which are pollinated by wild, non-domesticated male trees. Field-grown carob trees, either young or mature, do not show symptoms of Fe deficiency in leaves in contrast to *Citrus* species cultivated in the same area. Moreover, its optimal growing conditions are found in calcareous, alkaline soils, i.e. it is a calcicole species (Correia and Martins-Loução, 2005).

Strategy I, found in dicots in response to Fe deficiency, includes biochemical changes with enhanced proton extrusion leading to acidification of the rhizosphere, greater activity of ferric chelate-reductase (FC-R) that convert Fe(III)-chelates to Fe(II), and more Fe(II) transporters that allows Fe to cross the root plasmalemma (Walker and Connolly, 2008).

Few studies have compared calcicole species with those sensitive to Fe deficiency. In a comparative study of two pear rootstocks, Ma et al. (2006) found that *Pyrus xerophila* Yü, a wild rootstock adapted to calcareous soils in China, showed higher values of FC-R compared to *P. betulaefolia* Bunge (used as the rootstock for the Japanese pear) when bicarbonate was added to a nutrient solution with 100 μM Fe-EDTA.

The hypothesis we tested was that carob trees, being well adapted to alkaline calcareous soils, would have developed specific mechanisms in order to overcome the detrimental effects of these soils on Fe availability and use by plants. By comparing with a non-tolerant genotype, like *Poncirus*, grown under the same conditions, it should be possible to contrast the response of the enzyme FC-R in two genetic materials. The main objective was therefore,

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to study key-parameters involved in this abiotic stress in order to reveal the strategy of "efficient-iron plants".

2. Materials and methods

The experiment was conducted in a glasshouse and one-year old plants of *C. siliqua* L. ('wild' type) and *P. trifoliata* (L.) Raf. rootstocks were transferred from NPK fertilized turf, to polystyrene boxes containing 20 L of a half-strength Hoagland's nutrient solution with the following composition (in mM): 2.5 Ca $(NO_3)_2{\cdot}4H_2O$, 2.5 KNO_3, 0.5 KH_2PO_4, 1.0 $MgSO_4{\cdot}7H_2O$, and (in μM) 23.0 H_3BO_3, 0.4 $ZnSO_4{\cdot}7H_2O$, 0.2 $CuSO_4{\cdot}5H_2O$, 4.5 $MnCl_2{\cdot}4H_2O$ and 1.0 MoO_3. Iron was added to the solutions as Fe(III)-EDDHA at three different concentrations (in μM), 0 (Fe0), 1 (Fe1) and 10 (Fe10) for *C. siliqua*, and 0 (Fe0), 1 (Fe1) and 40 (Fe40) for *P. trifoliata*, since preliminary observations indicated that 10 μM Fe was insufficient for *P. trifoliata*. The pH of the solutions was adjusted to 6.0 ± 0.1. At the beginning of the experiment, the electrical conductivity (EC) of the solution was 1.20 dS m^{-1}, and this was monitored periodically so that the solutions were changed when the value was less than 1.10 dS m^{-1}.

During the experimental period, plants were grown under natural photoperiod conditions and air temperature ≤25 °C. There were 10 replications (plants) per 20-L container, in a total of 30 plants (three containers) per treatment and each rootstock. The containers were distributed in a complete randomized design.

The shoot height was measured in all plants of each treatment at the beginning and at the end of the experiment, and to compare the two plant species, the relative growth rate (RGR) was subsequently calculated as described by Pestana et al. (2011). Total leaf chlorophyll was estimated using the portable SPAD-502 meter (Minolta Corp., Japan) in fully expanded young leaves of both species.

The activity of root FC-R (EC 1.16.1.17) was measured by the formation of the Fe(II)-bathophenantrolinedisulfonate (BPDS) complex from Fe(III)-EDTA (Bienfait et al., 1983). Measurements were performed 50 days after the beginning of the experiment, with one root tip excised with a razor blade from each plant. Each excised root tip (approximately 2 cm) was incubated in an Eppendorf tube in the dark with 900 μL of micronutrient-free half Hoagland's nutrient solution, containing 300 μM BPDS, 500 μM Fe(III)-EDTA and 5 mM MES, pH 6.0. Readings were done after centrifugation, one hour after starting the incubation. An extinction coefficient of 22.14 mM cm^{-1} was used. Blank controls without root tips were also used to correct for any unspecific Fe reduction.

The effects of Fe treatments were evaluated by one-way analysis of variance and the means compared using the Duncan Multiple Range Test (DMRT) at $P < 0.05$ (SPSS software version 17.0).

3. Results

At the beginning of the experiment, carob and *P. trifoliata* plants had a height of about 15 cm and 20 cm, respectively. SPAD readings in the mature leaves were about 44 and 59 for carob and *P. trifoliata*, respectively. *P. trifoliata* plants of the Fe40 treatment showed the highest RGR of 12 mm per day compared to other treatments (Fe0 and Fe1). On the other hand, carob plants kept a low and constant RGR of 2 mm per day, irrespective of Fe levels in nutrient solution (Fig. 1A).

At the end of the experiment only *P. trifoliata* plants grown under total Fe depletion (Fe0) or with low levels of Fe (Fe1) showed symptoms of Fe chlorosis, with SPAD values (Fig. 1A and B) of 9% and 13%, respectively, of the values of plants grown with 40 μM Fe. In contrast, SPAD readings of young carob leaves remained high in all treatments (Fig. 1B) without evident symptoms of Fe chlorosis.

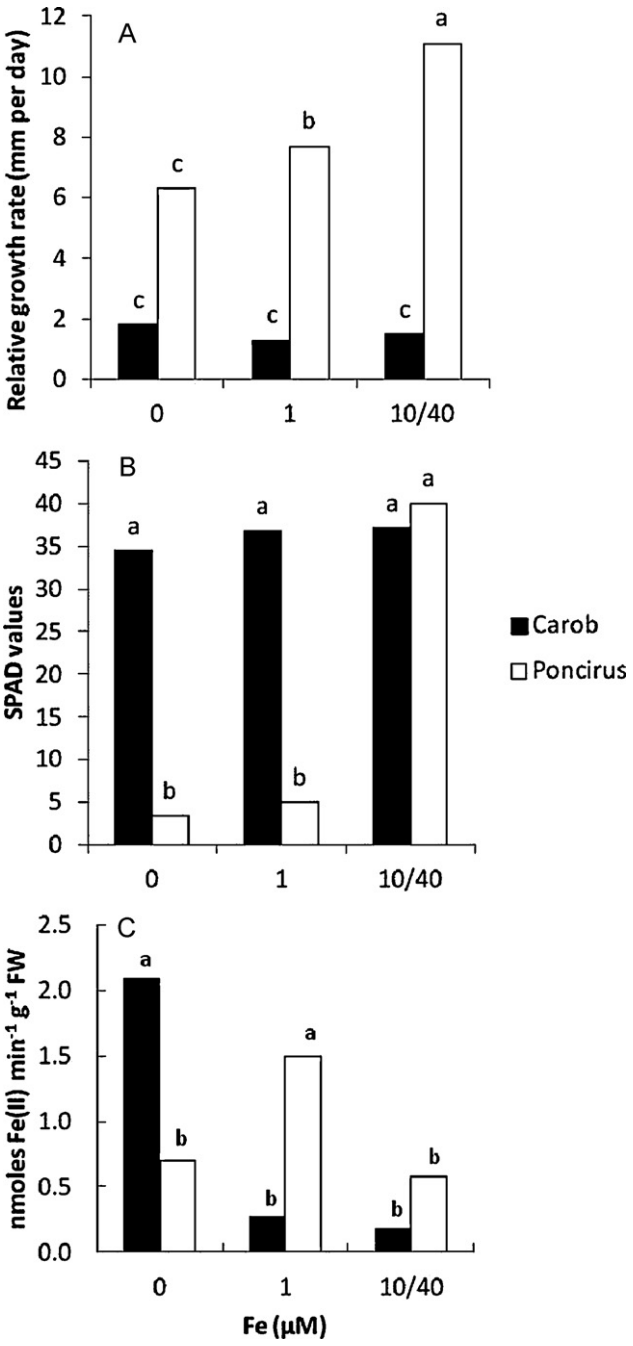

Fig. 1. Relative growth rate (A), mean SPAD values (B) and FC-R activity (C) determined at the end of the experiment (after 50 days) in each treatment and plant species. In each graph, columns with different letter indicate significant differences at $P < 0.05$ (Duncan Multiple Range Test).

The highest FC-R activity (Fig. 1C) in *P. trifoliata* was obtained in the Fe1 treatment, while plants of Fe40 and those grown without any Fe in the nutrient solution had lower FC-R activities. A different response was observed in carob roots, since high activity of FC-R was only observed in the Fe0 treatment.

4. Discussion

Plants of both species growing with high levels of Fe remained green during all the experimental period, and SPAD values were within the normal range observed in Citrus (Pestana et al., 2005) and carob rootstocks (Correia et al., 2003) grown in hydroponics.

Chlorotic plants with SPAD values bellow 5.0 indicate a strong decrease of leaf chlorophyll, an inefficient photosynthetic apparatus (Pestana et al., 2001) and, consequently, a small growth rate.

In contrast, carob plants grown for the same period of time (50 days) did not show symptoms of Fe deficiency even when grown with total depletion of Fe. In agreement with this, carob plants of all treatments had similar SPAD and RGR values at the end of the experiment. This means that under Fe depletion, leaf chlorophyll in newly formed leaves was ensured by Fe endogenous pools and an efficient translocation.

The differences observed between these species may be partially explained by the slow growing pattern of carob. In a recent comparative study of several Citrus rootstocks, Pestana et al. (2011) demonstrated that in Sour orange, growth rates were small and this was suggested as a strategy to explain the high degree of tolerance to Fe deficiency. Slow growing species should have smaller demands for nutrients, including Fe. Plants adapted to grow with shortage of nutrients are expected to conserve them (Lambers et al., 2008) and it is possible to presume that carob follows a conservative-type strategy.

Another key factor for the contrasting responses in both species was the different pattern of FC-R activity. There are a large number of studies demonstrating an increase of root FC-R in plants exhibiting Fe deficiency symptoms but the requirement of small amounts of Fe for FC-R has also been described in several species (e.g., Pestana et al., 2004; Abadía et al., 2011). Carob plants grown without any Fe (Fe0) had a high FC-R activity and no leaf chlorosis. Elevated Fe(III) reducing rates are related to higher tolerance to Fe stress (Castle et al., 2009) and several genes that are differentially overexpressed in Fe deficiency conditions were already identified in *P. trifoliata* (Forner-Giner et al., 2010). The high activities of FC-R may be deactivated by Fe-resupply as demonstrated by López-Millán et al. (2001). In carob we may conclude that after 50 days under total depletion of Fe in the solution (Fe0), the high FC-R activity may be considered as a response mechanism which can be an opportunity to take up greater amounts of Fe.

Since no external Fe was added to Fe0 carob plants during the 50 days of the experiment, a plant signal (endogenous Fe cannot be discarded) induced the higher FC-R activity. It is reasonable to admit that when carob plants are under severe Fe deficiency their growth is reduced to maximize the Fe-uptake mechanism (i.e. higher FC-R). In the sensitive *Poncirus*, on the other hand, a similar behaviour was observed but only if small amounts of Fe were present in the solution (Fe1). In this case, a less conservative strategy was found as the lack of Fe rapidly affected chlorophyll synthesis.

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Relationships between strawberry fruit quality attributes and crop load

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Vegetative growth

ABSTRACT

Crop load can influence fruit quality in several horticultural species. The aim of the present study was to determine the effect of different concentrations of calcium on crop quality traits in three short-day strawberry (*Fragaria* × *ananassa* Duch.) cultivars ('Ventana', 'Camarosa' and 'Candonga') and to assess the relationships between crop load and quality parameters. The studies were conducted using a hydroponic system in a greenhouse. Calcium was added as $Ca(NO_3)_2$ at 2 mM, 3 mM, 4 mM and 5 mM. A completely randomized block design (4 Ca concentrations × 3 cultivars) with three replicates was used. Each replicate consisted of 12 plants grown in polyethylene bags (100 cm × 18 cm × 3 cm) filled with coconut peat. Titratable acidity, total soluble solids and firmness were measured throughout the experimental period. Calcium application had no effect on fruit quality attributes but the genotype effect was clear. At the end of the experiment (28th May, 2008), titratable acidity was positively related to the fresh weight of above-ground biomass and number of leaves respectively in the 'Ventana' and 'Camarosa' cultivars. Higher values of total soluble solids were found at low crop load in 'Ventana' but in 'Camarosa' this relation was not found. In 'Candonga', higher total soluble solids were linked to crop load. In 'Ventana', titratable acidity significantly decreased as crop load increased, and in 'Camarosa' high values of titratable acidity were found at different values of crop load. 'Ventana' seemed to be more sensitive to the effects of crop load patterns. Genotype was an important factor in determining fruit quality parameters.

1. Introduction

Calcium (Ca) is a nutrient that differs from others as it appears in fleshy fruit only in small amounts, and far less than in leaves. The role of Ca in the regulation of fruit maturation and ripening processes is well-established (Ferguson, 1984). Ca is one of the most important nutrients involved in fruit ripening specifically because of its role in cell wall strengthening and membrane function (Poovaiah et al., 1988). Improving fruit Ca concentrations is often difficult to achieve. Attempts to increase Ca fruit levels have not always been successful and the results are often contradictory (Roy et al., 1999; Joyce et al., 2001). It is known that low fruit Ca content may lead to physiological and pathological disorders, and the fruits affected usually have a short shelf life (Fallahi et al., 1997). As a result, Ca is applied before and after harvesting to prevent physiological disorders, delay ripening and improve the quality of fruits crops, including the strawberry (Asrey et al., 2004; Dunn and Able, 2006; Hernández-Muñoz et al., 2006). However, while postharvest Ca treatments can be effective in raising fruit Ca levels in apples, the effectiveness of preharvest Ca sprays is less certain (Fallahi et al., 2010). In apples, sprays of soluble Ca reduce the incidence of bitter pit but do not always increase Ca concentration in cortical tissue (Fallahi et al., 2010). In strawberries, Ca is implicated in some fruit physiological disorders (Sharma et al., 2006) but Palencia et al. (2010) observed that the incidence of tipburn was also related to foliar K:Mg and K:Ca ratios.

It is well-known that consumers now pay much more attention to food quality traits. Nutritional value of strawberry fruits is demanded by growers and consumers for general health benefits and quality can be described by several parameters, including antioxidant capacity (Capocasa et al., 2008). Strawberry fruits is a source of micronutrients and phenolic compounds, most of which are natural antioxidants and contribute to a high nutritional quality (Roussos et al., 2009; Tulipani et al., 2011). Consumers also prefer sweet strawberries, and sweetness is positively correlated to soluble solid content. Fruit soluble solids and titratable acidity (TA) are quantitatively inherited (Shaw, 1990), and Keutgen and Pawelzik (2007) reported that decreasing soluble solid content in strawberries results in lower consumer acceptance of fruits.

Strawberry plant morphology is affected by cultivation practices, and plant size is related to fruiting potential. The stored assimilates in the crowns and roots have been reported to improve strawberry plant performance after a period in cold storage (Lieten

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et al., 1995). Whitehouse et al. (2009) proposed manipulating the production pattern of two strawberry cultivars by defoliating the plants, and therefore changing the normal course of source to sink pathways. The cropping pattern was changed, but cultivars respond differently to defoliation treatments.

Crop load (CL) may influence fruit quality in certain horticultural species. In apples cv. 'Jonagold', Stopar et al. (2002) found that lower CL (expressed as the number of fruits per crown) increased total polyphenols, but this response was not observed in other cultivars (Unuk et al., 2006). In peaches, increased CL negatively affected fruit soluble sugars and titratable acidity (TA), but the result was dependent on the scion/rootstock combination (De Salvador et al., 2007). It is possible to assume that any increase in the plant's biomass production (such as the number of leaves or fresh weight) may change the nutritional allocation patterns in fruits, with direct implications for crop quality. Therefore, quality parameters may change according to CL seasonal variations, an issue which, to our knowledge, yet to be studied in strawberries.

The aim of the present study was to determine the effect of different concentrations of Ca on fruit quality traits in three short-day strawberry (*Fragaria* × *ananassa* Duch.) cultivars ('Ventana', 'Camarosa' and 'Candonga') and to assess the relationships between CL and fruit quality parameters.

2. Materials and methods

The studies were conducted in a transparent polyethylene greenhouse measuring 160 m^2 at the Gambelas *Campus* of the University of Algarve, Portugal (7°58′W, 37°02′N) from October 2007 to May 2008. Three different short-day strawberry cultivars ('Ventana', 'Camarosa' and 'Candonga') were grown in polyethylene bags (100 cm × 18 cm × 3 cm) containing coconut peat (Pelemix Spain, S.L., Murcia-Spain), in an open soilless growing system. The polyethylene bags were mounted on support structures at a height of 100 cm and were watered by a drip irrigation system with one dripper per bag delivering 8 L h^{-1}. A complete concentrated fertilizer solution (without Ca) was injected into the irrigation system from a stock tank throughout the growing season. The nutrient solution consisted of (mg L^{-1}): N 271, P 702, K 586, Mg 207, S 414, Fe 8, Mn 4, Cu 0.3, Zn 0.8, B 0.7 and Mo 0.3, in accordance to the standard crop cultivation practices (Palencia et al., 2010).

Each cultivar was fed with four different Ca concentrations (2 mM, 3 mM, 4 mM and 5 mM) supplied as $Ca(NO_3)_2$. The smallest Ca concentration (2 mM) corresponded to that of the irrigation water. Additional Ca was applied using inverted glass bottles (1 L of calcium nitrate) placed 30 cm above the bags. These solutions were applied once per week and each bottle was replenished just before the next application.

Ripe fruits from each treatment (cultivar × Ca concentration) were harvested throughout the period of the experiment. Yield per plant were also calculated. Harvested fruits per bag were graded into two commercial classes: class-1 (fresh weight ≥22 g per fruit) and class-2 fruits (fresh weight <22 g per fruit). The first sampling was taken in February 2008 and the last in May. At each sampling date, all fruits from each bag and treatment were gathered for quality assessment and converted into pulp using a mixer. TA expressed as g of citric acid 100 g^{-1} (fresh weight) was measured in each treatment by titrating 10 g of the pulp plus 10 mL of H_2O with 0.1 mol L^{-1} NaOH up to pH 8.1. Total soluble solids (TSS, expressed as °Brix) was determined using an automatic temperature-compensated PR101 digital refractometer (Atago Pallette PR101). Firmness was evaluated in a sub-sample of 3–4 fruits from each treatment and in four sampling dates using a portable penetrometer. Results were expressed in g cm^{-2}.

The number of leaves (NL) was registered throughout the period of the experiment in previously selected plants from each treatment. CL was calculated as the ratio between yield and the NL per plant, registered on the same date or on the closest date. Since fruit thinning was not done, the patterns obtained correspond to natural crop behaviour. At the end of the experiment (28th May, 2008), plants from each treatment were removed from the bags and the total fresh weight of the above-ground biomass (leaves and crowns) was recorded.

The experimental design was a complete randomized block (3 replicates × 4 Ca concentrations × 3 cultivars). Each replicate consisted of one polyethylene bag with 12 plants. The main effects (Ca level and cultivar) on quality parameters were evaluated by variance analysis. Means were compared using Duncan's multiple range test with a significance level at 5%. Linear models were used to describe the relationships between vegetative (NL and biomass) and fruit quality parameters at the last harvesting date (28th May). The best-fitted models were chosen with regard to the variation of quality parameters due to CL values. All data analysis was carried out with the SPSS program version 17.0.

3. Results

At the end of the experiment, NL was similar in 'Camarosa' and 'Ventana'. Lower NL was found in 'Candonga' (Table 1) but with no statistical significance.

Total yield per plant was 172 g, 93 g and 130 g respectively for 'Ventana', 'Camarosa' and 'Candonga' (Table 1). The 'Ventana' cultivar had the highest accumulated production in cycle (24.8 kg). Also, a greater percentage of heavier fruits (class-1) were found in 'Ventana' compared to other cultivars.

TA was different between cultivars but similar between Ca treatments (Table 2). Lower values were obtained in 'Ventana' plants in February, March and May, compared to other cultivars. TA varied from 0.24 ('Ventana'-26th March) to 0.42 ('Camarosa'-28th May). The highest TA level was obtained from 'Camarosa' and 'Candonga' fruit. Regarding TSS, Ca application had no effect despite the small differences observed on the first sampling date (Table 3). 'Ventana' fruits had lower TSS values on several sampling dates compared to 'Candonga' and 'Camarosa' (Table 3). TSS ranged from 6.23 °Brix ('Ventana'-22nd February) to 10.35 °Brix ('Camarosa'-14th March). The effect of Ca treatments on fruit firmness remained unclear (Table 4). However, 'Ventana' fruits were less firm than 'Candonga' and 'Camarosa' fruits. It appears that the effect of genotype on fruit quality parameters is more important than Ca application.

In order to look if a relationship exists between fruit quality parameters and vegetative growth, several regression models were

Table 1
Number of leaves and yield per plant of three cultivars, pooling together the calcium treatments at the end of the experiment (28th May, 2008). Percentage of class-1 and class-2 fruits is also shown. SD: standard deviation.

Cultivar	Number of leaves (NL) ± SD	Yield per plant (g $plant^{-1}$)	Distribution of fruits per class (%)	
			Class-1	Class-2
'Ventana'	22.3 ± 2.8	172	26.0	74.0
'Camarosa'	22.3 ± 5.3	93	7.4	92.6
'Candonga'	21.8 ± 1.8	130	10.5	89.5

Table 2
Titratable acidity (g citric acid 100 g^{-1} fresh weight) between cultivars and calcium treatments throughout the period of the experiment. In each column, means with the same letter indicate no significant differences between cultivars or Ca treatments at $P < 0.05$. ns: not significant.

Cultivar	22 February	14 March	26 March	23 April	14 May	28 May
'Ventana'	0.25 b	0.27 b	0.24 c	0.32 ns	0.36 b	0.38 b
'Camarosa'	0.29 a	0.28 b	0.29 b	0.35 ns	0.42 a	0.42 a
'Candonga'	0.30 a	0.31 a	0.32 a	0.33 ns	0.40 a	0.36 b
Calcium (mM)						
2	0.28 a	0.39 ns	0.29 ns	0.34 ns	0.41 ns	0.37 ns
3	0.29 a	0.30 ns	0.29 ns	0.31 ns	0.37 ns	0.40 ns
4	0.30 a	0.29 ns	0.29 ns	0.34 ns	0.40 ns	0.38 ns
5	0.25 b	0.28 ns	0.28 ns	0.32 ns	0.40 ns	0.38 ns

Table 3
Total soluble solids (°Brix) between cultivars and calcium treatments throughout the period of the experiment. In each column, means with the same letter indicate no significant differences between cultivars or Ca treatments at $P < 0.05$. ns: not significant.

Cultivar	22 February	14 March	26 March	23 April	14 May	28 May
'Ventana'	6.23 b	6.85 b	7.24 b	8.65 ns	8.35 b	9.75 ns
'Camarosa'	8.50 a	10.35 a	8.44 a	9.53 ns	8.35 a	9.24 ns
'Candonga'	7.10 a	9.96 a	8.46 a	8.79 ns	8.86 a	9.90 ns
Calcium (mM)						
2	7.18 ab	9.10 ns	8.22 ns	9.15 ns	8.47 ns	9.58 ns
3	7.97 a	9.08 ns	7.89 ns	8.87 ns	8.50 ns	9.73 ns
4	7.69 ab	9.09 ns	7.95 ns	8.59 ns	8.88 ns	10.01 ns
5	6.32 b	7.60 ns	8.14 ns	8.89 ns	8.27 ns	9.60 ns

Table 4
Firmness (g cm^{-2}) between cultivars and calcium treatments throughout the period of the experiment. In each column, means with the same letter indicate no significant differences between cultivars or Ca treatments at $P < 0.05$. ns: not significant.

Cultivar	29 February	14 March	30 April	28 May
'Ventana'	275.6 b	286.1 b	274.7 c	249.9 b
'Camarosa'	321.8 a	338.9 a	361.6 a	280.3 a
'Candonga'	347.4 a	326.3 a	310.1 b	288.5 a
Calcium (mM)				
2	330.5 ns	302.6 ab	317.0 ns	252.1 b
3	286.1 ns	294.3 b	319.1 ns	308.7 a
4	313.3 ns	329.6 a	321.9 ns	258.9 b
5	323.9 ns	321.5 ab	307.4 ns	266.9 b

tested for each cultivar (Table 5) at the end of the growing season (28th May), when the crop reaches its maximum vegetative vigour. In 'Ventana', TA was positively related to the fresh weight of above-ground biomass (leaves and crown). In 'Camarosa', higher TA values were observed in plants with higher NL ($r^2 = 0.97$). This trend was also observed in 'Candonga' but was not significant ($r^2 = 0.39$; $P = 0.055$). In this cultivar, TSS was inversely related to biomass fresh weight, which means that higher TSS in fruits is related to less biomass. To analyse in more detail the possible effect of growth on the quality of the fruits, the variation of the CL was studied considering all Ca treatments as one. In Fig. 1, CL decreases over time in 'Ventana' and 'Camarosa', but in 'Candonga' CL was lowest on the first sampling date (56 days after the transplanting) originating a non-linear response. As shown in Fig. 2, higher TSS is associated to low CL in 'Ventana' plants. In 'Camarosa', no relation was found, as TSS was kept constant throughout the season. In 'Candonga', lower

Table 5
Linear regression models for each cultivar at the end of the season (28th May).

'Ventana'	r^2	N	P
TSS = −0.0004 × NL + 9.757	0.00	10	0.991
TA = 0.0016 × NL + 0.3554	0.23	10	0.157
TSS = −0.0001 × FW + 9.758	0.00	10	0.991
TA = 0.0009 × FW + 0.3384	0.51	10	0.020
'Camarosa'			
TSS = −0.3380 × NL + 18.015	0.48	5	0.193
TA = 0.0150 × NL + 0.034	0.97	5	0.002
TSS = 0.0286 × FW + 6.879	0.09	5	0.630
TA = 0.0006 × FW + 0.3778	0.03	5	0.767
'Candonga'			
TSS = −0.236 × NL + 14.875	0.36	10	0.068
TA = 0.0065 × NL + 0.2321	0.39	10	0.055
TSS = −0.1072 × FW + 15.910	0.40	10	0.048
TA = 0.0013 × FW + 0.2948	0.08	10	0.421

NL: number of leaves; TSS: total soluble solids; FW: fresh weight of above-ground matter (leaves plus crown); TA: titratable acidity.

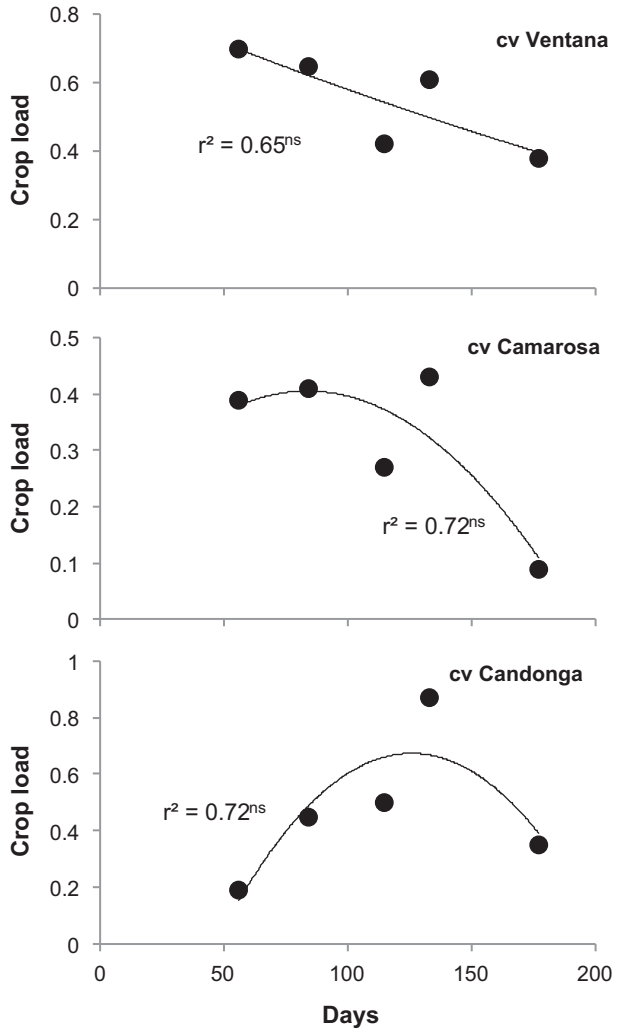

Fig. 1. Seasonal variation of crop load of the cultivars studied. ns: non-significant.

CL was not related to higher TSS as in 'Ventana' but a significant trend was recorded. TA is also influenced by CL (Fig. 3). In 'Ventana' higher TA is associated to low CL, but this relationship is not found in 'Candonga'.

4. Discussion

The ripening stage of strawberries at harvest date is crucial since it enables delivery of fruits to consumers in their best condition in terms of nutritional, sensory and functional properties. TA, TSS and fruit firmness are frequently used to reach this optimal point of fruit maturity for harvest. It is also known that the chemical composition of fruits significantly changes according to cultivar and stage of maturity (Sturm et al., 2003).

In this study, TA values were lower than those reported by Kafkas et al. (2007) in nine strawberry hybrids and two cultivars ('Camarosa' and 'Osmanli'), but these plants were grown under soil conditions. Perkins-Veazie (1995) found that TA varies from 0.45 to 1.81%, and TSS range from 4 to 11 °Brix depending on the strawberry cultivar, among other factors. In general, 'Camarosa' and 'Candonga' cultivars presented higher values of TSS compared to 'Ventana'. These TSS values were also higher than those obtained by Sturm et al. (2003) working with 12 other strawberry cultivars (average TSS: 6.7%) but similar to those reported by Roussos et al. (2009) for 'Camarosa' fruits. The major effect observed on fruit

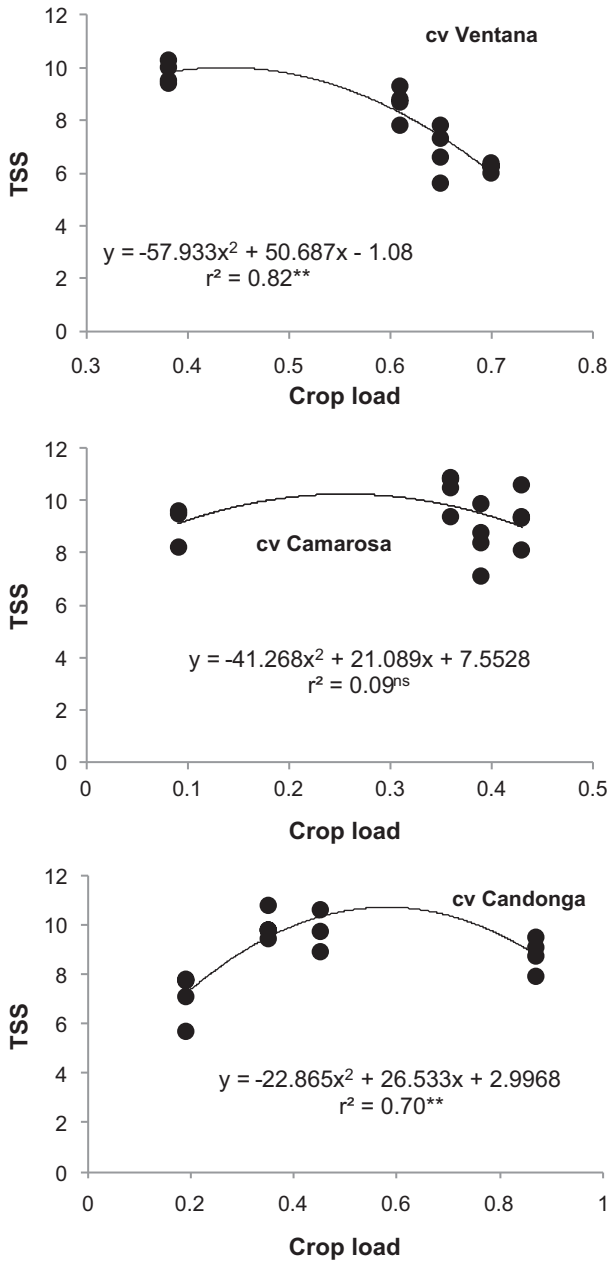

Fig. 2. Relationship between TSS (°Brix) and crop load (CL) throughout the crop 2 cycle. **Significant at $P < 0.01$; ns: non-significant.

quality, as for TA and TSS values, could be related to the cultivars used.

In our experiment, Ca had no significant effect on fruit quality as previously observed in a concurrent study (Palencia et al., 2010) where Ca treatments did not affect tipburn incidence, a nutritional disorder associated to Ca deficiency.

Several authors (Alcaraz-López et al., 2003; Wójcik and Lewandowski, 2003) found a significant relation between fruit firmness and Ca content. However in this study, firmness tended to decrease during the growing season in all cultivars despite the different Ca treatments. The variability of values for firmness between harvest dates was also reported by Palha et al. (2009) for 'Camarosa' and may be related to the increase in environmental and fruit temperature, leading to a loss of firmness in strawberry fruits (Olías et al., 1995). This trend was found in all cultivars; however, 'Ventana' fruits were less firm than those of 'Candonga' and 'Camarosa'

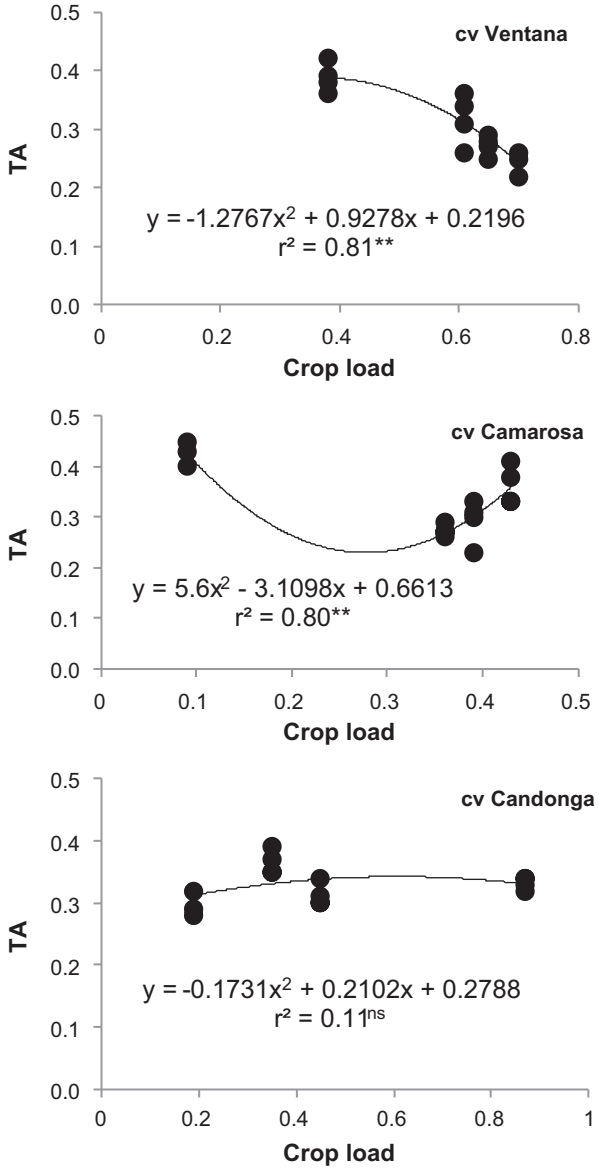

Fig. 3. Relationship between titratable acidity (TA) and crop load throughout the crop cycle. **Significant at $P < 0.01$; ns: non-significant.

which were similar in firmness, indicating a clear genotype effect. Recently, Tulipani et al. (2011) have also confirmed a relevant genotype-dependent response to environmental stress conditions.

The relation between vegetative growth parameters and fruit quality is supported by the positive trends of the models presented in this study: fruit TA was positively and significantly related to the fresh weight of above-ground biomass (in 'Ventana') or to NL (in 'Camarosa'). Recently, Crespo et al. (2010) have shown that the leaf area per plant and yield were significantly affected by the different altitudes of the experimental sites. Previously, Carlen et al. (2007) also reported a positive correlation between the leaf area/yield ratio of strawberry cultivars and their fruit TSS.

Significant reductions in CL have been associated to increasing in fruit TSS (Whiting and Lang, 2004; Neilsen et al., 2007; Marsal et al., 2009). In apples cv. 'Jonagold', the concentration of all phenolic compounds in fruit was inversely related to CL (Stopar et al., 2002) but this response was not found in apple cv. 'Golden delicious' (Unuk et al., 2006). In the 'Suncrest' peach cultivar, fruit TSS and TA were negatively affected by increasing CL (De Salvador et al., 2007). In grapevines, the fresh weight of bunches and berries, and the TSS of berries decreased as a result of heavier fruit loads (Morinaga et al., 2003). There is some evidence that an appropriate fruit load is important to maintain high fruit quality. Carbon-based compounds are very important for fruit growth, and the supply of carbon to the fruit may be limited during early fruit development due to competition arising from the demand of too many sinks (Link, 2000).

In our experimental conditions, the highest quality fruits (higher TSS and slightly higher TA) of the 'Ventana' cultivar were found in late season, when the plant increases the supply of assimilates (C compounds such as sugars) to highly demanding sinks. Citrus trees with a heavy CL had a lower branch sap flow rate, and lower juice TSS (Yonemoto et al., 2004). In strawberry genotypes from mountain regions where plants produced higher fruit yield over a shorter period, the concentration of vitamin C was negatively related to the average yield per day (Crespo et al., 2010). In 'Camarosa', despite the decrease in CL throughout the crop cycle TSS was kept constant, thus explaining the lack of significance in the models tested. 'Camarosa' is a widely cultivated and highly productive cultivar (Antunes et al., 2010) which may indicate an efficient supply of photoassimilates to fruits, therefore overcoming the competitive effect of vegetative sinks.

In this work, vegetative growth was related to the quality of fruits and fruit quality parameters were influenced by CL throughout the season. Genotype is the major factor in determining nutritional quality in fruit, but is also affected by crop conditions (environmental and cultivation techniques), the ripening season, pre- and postharvest conditions, shelf life and processing (Connor et al., 2002). Regarding berry fruits, recent research confirms the role of genotype as the main source of variation in anthocyanins and sugar content (Crespo et al., 2010).

By inhibiting vegetative growth (hormonal treatments or leaf removal), the allocation of internal resources may change and fruit quality may be improved, but this approach needs further research into a wide range of cultivars and environmental conditions. The results of this work confirm the strong effect of plant genotype on CL and that fruit quality of marketable yield may change according to the existing sinks. The fruit quality traits of 'Ventana' (TSS and TA) seem to be highly sensitive to CL variation, which means that this cultivar is less able to adjust fruit quality to fruit-leaf imbalances. However, CL may be used by plant breeders and growers to manipulate source-to-sink pathways and thus reinforce fruit quality. By contrast, 'Camarosa' and 'Candonga' cultivars behaved differently suggesting that some fruit traits are quite dependent on the fruit-leaf balance.

The effect of genotype on strawberry nutritional quality is stronger than growing conditions (Capocasa et al., 2008), but our work supports the fact that within each cultivar vegetative growth patterns may play an important role in the development of fruit quality traits.

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Relationship between tipburn and leaf mineral composition in strawberry

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ABSTRACT

Malformation of emerging leaves with distortion of leaf tips, a condition known as tipburn, is frequently observed in strawberry. Calcium (Ca) deficiency has been considered the main cause of tipburn. The aim of the present study was to analyse the relationship between leaf mineral composition and the incidence of tipburn in three short-day strawberry (*Fragaria x ananassa* Duch.) cultivars ('Ventana', 'Camarosa' and 'Candonga') submitted to different concentrations of Ca. The studies were conducted in a hydroponic system in a greenhouse. Calcium was added as $Ca(NO_3)_2$ at 2 mM, 3 mM, 4 mM and 5 mM. A completely randomized block design (4 Ca concentrations × 3 cultivars) with three replications was used. Each replicate consisted of 12 plants grown in a polyethylene bag (100 cm × 18 cm × 3 cm) filled with coconut peat. Crown diameter and tipburn incidence were evaluated throughout the experimental period, and at the end of the experiment leaf mineral composition was assessed. In general, plants with larger crown diameters had a greater incidence of tipburn. The 'Candonga' cultivar had the smallest incidence of tipburn, while the 'Camarosa' and 'Ventana' cultivars were more susceptible. There was no correlation between level of Ca applied and incidence of tipburn. The incidence of tipburn was associated with foliar K:Ca and K:Mg ratios. Ratios above 3.40 for K:Mg and 1.77 for K:Ca represented a risk of more than 50% of tipburn incidence, when overall means for all cultivars and levels of Ca were used.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is grown throughout the world and its production and growth areas increase each year. Spain is the world's second-largest strawberry producer after the USA, and in the south of Portugal most of the strawberry production is conducted in hydroponics system.

Leaf tipburn in strawberries is a physiological disorder which may cause serious economic losses (Brumm and Schenk, 1993; Wissemeier, 1996). It is first visible to the naked eye as a water-soaked greyish area at the tips of emerging leaves. These damaged areas subsequently die and thus the expansion of unaffected leaf tissues behind the tip is restricted, causing leaflets to become crinkled and distorted.

The susceptibility to tipburn is genetically determined (Lineberry and Burkhart, 1943) but it is influenced by environmental conditions (e.g. Maynard and Barker, 1972; Cox et al., 1976; Collier and Tibbitts, 1982; Wissemeier, 1996; Chow et al., 2004). Calcium deficiency is often considered as the main cause of tipburn. Lineberry and Burkhart (1943) and Johansen and Walker (1963) obtained tipburn in plants growing in sand cultures after withholding Ca. The incidence of tipburn in strawberry can also be promoted by fast plant growth (Saure, 1998), and by a large nitrate supply that stimulated plant growth (Brumm and Schenk, 1993). The leaves of plants developing tipburn are often larger and more succulent than leaves of non-affected plants (Palzkill et al., 1980).

Intense light and extended photoperiods, increase the occurrence and severity of tipburn (e.g. Gaudreau et al., 1994), while low temperatures may delay or prevent the onset of the disorder (Cox et al., 1976). A positive correlation between air humidity and the occurrence of tipburn was reported in lettuce (Barta and Tibbitts, 1986), cabbage (Wiebe, 1975; Palzkill et al., 1980), and cauliflower (Krug et al., 1972).

Recent studies on tipburn in the strawberry cultivar 'Camarosa' focused on the use of nutrient solutions with different proportions of Ca, Mg and K, while maintaining a constant concentration for the sum of the three cations (San Bautista et al., 2009). Nutrient solutions poor in K decreased tipburn incidence, while solutions rich in Mg or poor in Ca enhanced tipburn incidence. These authors explained these results based on the known antagonism between these cations. However, varying the amounts of K, Ca and Mg in the nutrient solution meant that levels of these nutrients could be

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Table 1
Leaf mineral composition at the end of the experiment (29th May).

Applied Ca (mM)	K	Mg	Ca	Cu	Zn	Mn
	g kg^{-1} dw			mg kg^{-1} dw		
'Ventana'						
2	23.7a	4.4a	9.2a	8.5a	40.6a	49.7a
3	22.5ab	3.6b	7.9bc	7.5a	35.1a	40.7ab
4	20.6b	3.6b	7.6c	8.4a	39.3a	35.1b
5	22.7ab	4.1ab	9.0ab	8.8a	37.3a	39.4ab
'Camarosa'						
2	21.7a	4.5ab	8.1ab	9.3a	31.9a	27.0a
3	21.3a	4.1b	7.4b	11.4a	28.6ab	28.4a
4	19.7a	4.1b	8.9a	6.2b	23.9ab	28.0a
5	14.7b	4.9a	8.4ab	4.5b	19.8b	30.5a
'Candonga'						
2	13.0b	4.8a	10.1a	5.4a	18.1a	33.0a
3	14.3ab	4.7a	9.5ab	6.4a	25.3a	41.3a
4	19.6a	4.6a	8.7ab	7.0a	20.1a	36.4a
5	19.9	4.1a	8.2b	7.0a	22.7a	35.4a
Cultivar	**	**	*	ns	**	**
Applied ca	ns	*	ns	ns	ns	ns
Cultivar × applied Ca	**	*	*	**	ns	ns

For each cultivar, means in a column followed by different letters denote significant differences among treatments, using the Duncan's test at $P < 0.05$; $n = 4$. Significance for the main effects (cultivar and level of applied) and interaction between factors are also shown; ns (non-significant).

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

outside the required range of concentrations. Therefore, to further investigate the importance of nutrient balance on the incidence of tipburn, in the present experiment we maintained the levels of K and Mg in the nutrient solution constant, and varied only the amount of Ca. We also expanded the previous study using three cultivars of strawberry, to take into account genetic differences in tipburn susceptibility so that results obtained could be more robust.

2. Materials and methods

The study was conducted on a polyethylene greenhouse with 160 m^2 in *Campus* of Gambelas, University of Algarve, Portugal (7°58′W, 37°02′N) from October 2007 to May 2008. Three different short-day strawberry cultivars ('Ventana', 'Camarosa' and 'Candonga') were grown in polyethylene bags (100 cm × 18 cm × 3 cm) containing coconut peat (Pelemix Spain, S.L., Murcia-Spain), in an open soilless growing system. The polyethylene bags were supported by metal structures (1 m high) and were watered with a drip irrigation system with one dripper per bag delivering 8 L h^{-1}. A concentrated complete fertilizer solution (without added Ca) was injected into the irrigation system throughout the growing season. The nutrient solution consisted of (mg L^{-1}): N 271, P 702, K 586, Mg 207, S 414, Fe 8, Mn 4, Cu 0.3, Zn 0.8, B 0.7 and Mo 0.3.

Each cultivar was fed with four different Ca concentrations (2 mM, 3 mM, 4 mM and 5 mM) supplied as $Ca(NO_3)_2$. The smallest Ca concentration (2 mM) corresponded to that of the irrigation water. Additional Ca was applied using inverted glass bottles (1 L of calcium nitrate) placed 30 cm above the bags. These solutions were applied once per week and each bottle was replenished just before the next application.

Each treatment (4 Ca concentrations × 3 cultivars) consisted of three polyethylene bags with 12 plants each on a completely randomized block design. Six plants were selected in each treatment. In these plants the crown diameter was measured and tipburn was assessed bi-monthly throughout the experimental period. Plants with symptoms of tipburn were counted and the percentage of tipburn incidence was calculated. At the end of experimental period (May) mature leaves were collected from the selected plants for mineral composition analysis. Plant material was dried at 75 °C and ground. Standardized procedures (A.O.A.C., 1990) were used to measure nutrient concentrations. Nitrogen was analysed by the Kjeldahl method. Other subsamples were ashed at 450 °C and digested with 10 ml HCl 1 M. Phosphorus was determined colorimetrically by the molybdo-vanadate method, and K, Mg, Ca, Fe, Mn, Cu and Zn were measured by atomic absorption spectrometry.

The main effects (Ca level and cultivar) on leaf mineral composition were evaluated by analysis of variance. Means were compared using the Duncan's multiple range test at 5% significance level. The best fitted model was used to describe the variation of the independent variables and the correlation coefficients were shown. Whenever possible, linear regressions were used. All data analysis was made with the SPSS program version 16.0.

3. Results

The concentrations of N, P and Fe in leaves of all cultivars were similar and not affected by the Ca level, with average values of 15.3 g kg^{-1} and 3.2 g kg^{-1} and 41.6 mg kg^{-1}, respectively. The concentrations of K, Mg, Ca, Zn and Mn differed between cultivars (Table 1), with the 'Ventana' cultivar having the greatest concentrations of K, Zn and Mn, and the 'Candonga' cultivar the greatest concentrations of Mg and Ca. Only leaf Mg was affected by Ca application, but there were also significant interactions cultivar × level of Ca for K, Mg, Ca, and Cu concentrations (Table 1).

Crown diameter correlated positively with the incidence of tipburn, considering all three cultivars and Ca treatments as a whole (Fig. 1). Although the correlation was not very strong, it still explained about 32% of the variation.

The incidence of tipburn increased notably between 80 and 120 days after the appearance of the first symptoms (described as day 0), but with differences between cultivars. The cultivar 'Ventana' (Fig. 2) had a period around 50–100 days after the appearance of tipburn when the symptoms practically disappeared only to increase later to practically 100% incidence. The cultivar 'Camarosa' had a positive correlation ($r^2 = 0.73$) between tipburn and time, and again the incidence was very large at the end of the experiment (Fig. 3). In contrast, the cultivar 'Candonga' was less susceptible to tipburn, and it was the only one in which incidence of tipburn was related to level of applied Ca at the end of the experiment (Fig. 4). However, no significant correlation was obtained between level of applied Ca

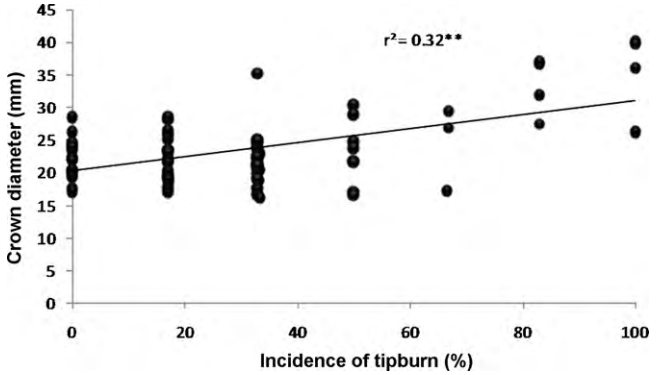

Fig. 1. Regression relationships of strawberry crown diameter (mm) and incidence of tipburn (%). **Significant at $P < 0.01$.

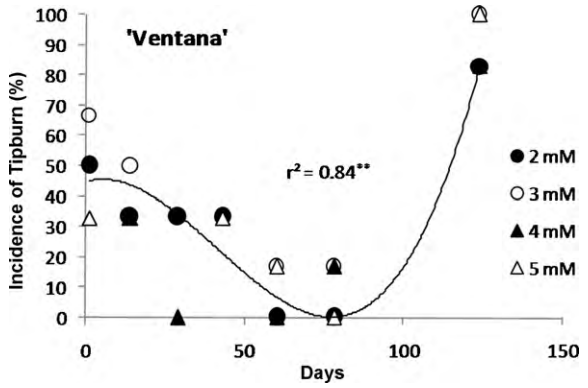

Fig. 2. Seasonal variation of the incidence of tipburn (%) in cv. 'Ventana'. **Significant at $P < 0.01$.

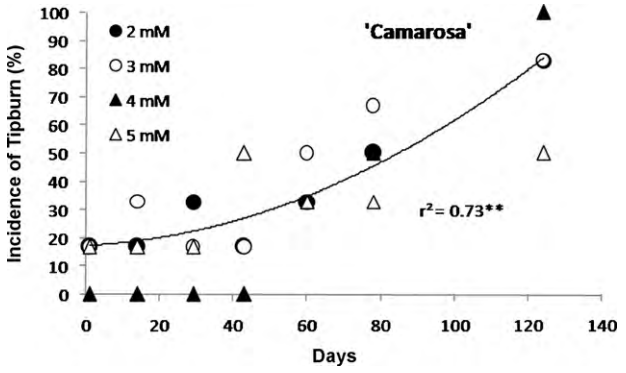

Fig. 3. Seasonal variation of the incidence of tipburn (%) in cv. 'Camarosa'. **Significant at $P < 0.01$.

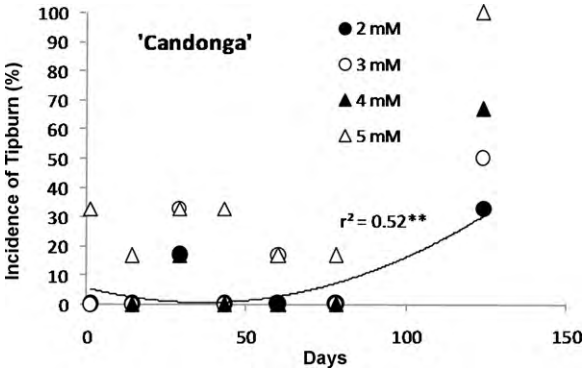

Fig. 4. Seasonal variation of the incidence of tipburn (%) in cv. 'Candonga'. **Significant at $P < 0.01$.

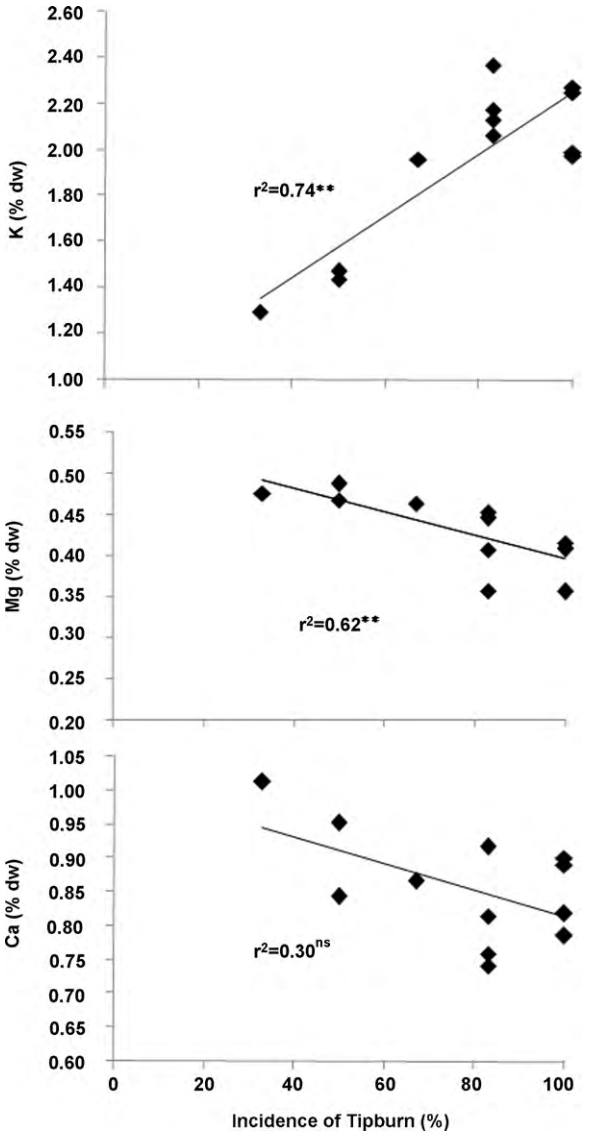

Fig. 5. Regression relationships between K, Mg and Ca leaf concentrations (g kg^{-1} dw) and incidence of tipburn considering all cultivars and all Ca concentrations at the end of the experiment (29th May). **Significant at $P < 0.01$; ns (non-significant).

and incidence of tipburn when values for all cultivars and dates of observation were used ($P = 0.07$; $r^2 = 0.30$).

At the end of the experiment, greater incidence of tipburn was positively related to K leaf concentration, and the opposite was true for Mg and Ca (Fig. 5). When the K:Ca and K:Mg were used, very strong correlations between these ratios and incidence of tipburn were obtained (Fig. 6). Assuming a threshold value of 50% of tipburn incidence, the response models will give a nutritional ratio of 3.40 for K:Mg and 1.77 for K:Ca. No correlations were found between any other nutrients and the incidence of tipburn.

4. Discussion

The results obtained in this experiment confirm previous observations by other authors but enlarge our understanding on the causes of tipburn.

Tipburn was only observed on young developing leaves as reported by several authors (e.g. Chow et al., 2004). Plants with larger crown diameters had a greater incidence of tipburn, showing a positive relationship between vegetative vigour of the crop

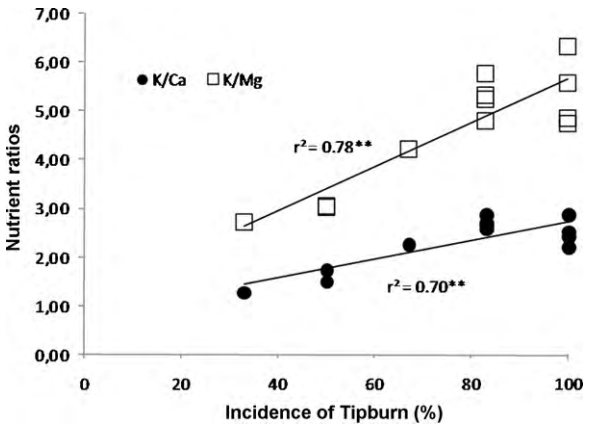

Fig. 6. Relationship between leaf K:Ca and K:Mg ratios and incidence of tipburn at the end of the experiment (29th May), considering all cultivars and all levels of applied Ca. **Significant at $P < 0.01$.

and the occurrence of tipburn as stated by Palzkill et al. (1980) and Saure (1998). A close analysis of this response shows that a greater tipburn incidence was observed during April–May in all cultivars, which corresponded to the most favourable growing conditions. As pointed out by Saure (1998), increased susceptibility to tipburn of stress-free growing plants (luxurious growth) seems to be caused by an enhanced level of gibberellins (GA). The mechanism is still a matter of controversy, but GA may reduce the stress tolerance by increasing the permeability of membranes or by impairing membrane integrity.

There were differences between cultivars on tipburn susceptibility, with cv. 'Candonga' being less susceptible to tipburn, with an incidence smaller than 35% until April. In a study with 'Candonga' and 'Camarosa' cultivars grown in a soilless system, San Bautista et al. (2008) also found that the cv. 'Candonga' was less susceptible to tipburn.

In a previous work, Palencia et al. (2008) observed that applied Ca concentrations had no significant effect on several vegetative parameters of 'Ventana', 'Candonga' and 'Camarosa' cultivars. In the present experiment, we show that changing the level of applied Ca from 2 to 5 mM did not lead to consistent increases in leaf Ca. More importantly, increasing the level of applied Ca did not decrease the risk of tipburn, as there was no correlation between the two when the overall data for all cultivars and sampling dates were pooled together.

Tipburn incidence was related to leaf Ca, Mg and K concentrations. Calcium was always within the sufficiency range, but its concentration was smaller in plants with tipburn, confirming the results of other authors (Bradfield and Guttridge, 1979; Chow et al., 2004). Calcium is required in large amounts and in an actively growing plant the Ca flux in the xylem is important but might be diverted to organs with large transpiration rates (White, 2001). If nights were cool, the intake of water and Ca to low transpiring organs (when guttation is observed) would minimize the risk of tipburn. In this work, under our greenhouse conditions, environmental conditions might have promoted tipburn.

Mason and Guttridge (1974) found a reduction of tipburn when the content of leaf Mg was decreased. In this experiment, the opposite trend was observed, although leaf Mg varied within a very small range (3.6–5.0 g kg^{-1}), leading to a gentle slope of the linear model, which may indicate a poor relationship of Mg concentration per se and incidence of tipburn.

Potassium concentration was greater in plants suffering from tipburn than in others. As pointed by Lieten (2006), K uptake may compete with Ca thus originating tipburn.

In the 'Camarosa' cultivar, San Bautista et al. (2009) found that tipburn was more frequent in plants growing in solutions with less Ca, but in the second year of experiment, the greatest incidence was observed in plants grown in solutions with more K. Strawberry has a larger demand for K. Although there was no over fertilization with K, a significant K uptake and translocation probably occurred due to the favourable growing conditions. In several cultivars of strawberry, Sharma et al. (2006) found that increased vigour was associated with overuse of N and K, which in turn, was associated with a greater incidence of albinism in fruits. In the present experiment tipburn incidence was closely associated with the balance between Ca, Mg and K, as shown by the strong correlations of tipburn incidence with K:Ca and K:Mg ratios. It is clear from our study that tipburn in strawberry does not result from an inadequate supply of Ca or from over fertilization with K. It is rather a conjugation of genetical susceptibility and environmental conditions, becoming more frequent as the growing season progresses. The 'Candonga' cultivar was less susceptible to tipburn and had the greatest Ca and Mg concentrations in leaves when grown in the same conditions as the other two cultivars.

For a particular cultivar, as the balance of K, Ca and Mg in leaves depends on transpiration rates, and this in turn is associated with temperature and humidity, the only way to prevent tipburn seems to be to bypass the xylem transport, with foliar applications of Ca and eventually Mg. In the long-term, breeding for cultivars that accumulate larger amounts of Ca and Mg in leaves would provide a permanent solution for this abiotic condition.

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Evaluation of Fe Deficiency Effects on Strawberry Fruit Quality

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Abstract

The effects of Fe deficiency on the antioxidant properties of strawberry juice was carried out with a day-neutral cultivar 'Selva'. Bare root transplants (without leaves) with approximately 18 cm, were transferred to Hoagland's nutrient solution with (Fe2.5) and without Fe (Fe0), using Fe-EDDHMA as the Fe source: 0 and 2.5 5 µM Fe. Plants were grown in 20 L containers in a glasshouse for 6 weeks (from April 27 to June 5) under natural light and air temperature ≤ 25ºC. Twelve transplants were used per treatment, distributed in a complete randomized design. Plants grown in absence of Fe revealed chlorotic symptoms approximately after three weeks, based on SPAD values measured in young leaves (‹20). The other treatments did not show any symptoms during the experiment. Fruits were harvested from each treatment, and juice was analysed for antioxidant activity by using the free radical α-α-diphenyl-β-picrylhydrazyl capacity (DPPH•), the Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays. In addition, fruits were analysed for total phenols and some organic acids. The phenolic content varied between 1251 and 1514 mg gallic acid equivalents (GAE) L^{-1} juice, respectively, in Fe0 and Fe2.5 treatments, but with no significant differences. Despite the same total soluble solids values in both treatments, it was found that Fe depletion reduced significantly the anthocyanins and total phenols of the fruits. However, ascorbic acid increased as well as antioxidant activity expressed by both DPPH and TEAC methods.

INTRODUCTION

Many studies have shown the importance of diets rich in antioxidants, which may help to prevent many diseases such as coronary heart disease and several types of cancer. Natural antioxidants, including phenolic compounds such as anthocyanins, vitamins, carotenoids etc., which come fruits and vegetables, are a better source of antioxidants than synthetic types (Miguel et al., 2007; Wang and Lin, 2000). Their main function is to neutralise free radicals in the human body and to avoid excessive oxidative stress involving reactive oxygen and nitrogen species (Ferreyra et al., 2007). Strawberries are a rich source of natural antioxidants, especially when consumed fresh (Montero et al., 1996). Iron deficiency, known as iron chlorosis, frequently occurs in Mediterranean regions where calcareous soils prevail (Pestana et al., 2003). It results in a decrease of photosynthetic pigments and symptoms are easily identified in young leaves as yellow coloured interveinal chlorosis. This deficiency decreases fruit yield, delays ripening and declines its quality (Álvarez-Fernández et al., 2003). Strawberry (*Fragaria ananassa* Duch.) production is seriously affected by induced Fe-deficiency. Kafkas et al. (2007) reported a decrease in fruit size and total soluble solids (TSS) content of strawberry plants

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with iron deficiency symptoms. Strawberry quality may be defined by texture, taste (soluble sugars and organic acids) and colour (anthocyanin content) at harvest (Kafkas et al., 2007). Although the main constituents of strawberries during maturation are well known, few studies have been carried out to investigate the effect of nutritional disorders on their content. The objective of the present study was to evaluate the effect of Fe deficiency on some chemical characteristics of strawberry fruits harvested at the same maturity stage.

MATERIALS AND METHODS

Bare root transplants (without leaves) with approximately 18 cm, were transferred to Hoagland's nutrient solution, with (2.5 μM Fe as Fe-EDDHMA) and without Fe. Plants were grown in 20 L containers in a glasshouse for 6 weeks (from April 27 to June 5) under natural light and air temperature ≤ 25ºC. Twelve transplants were used per treatment distributed in a complete randomized design. The first leaves emerged 15 days after the beginning of the experiment. Leaf chlorosis was determined using a SPAD-502 apparatus (Minolta, Osaka, Japan), and readings were done at least twice a week. Six readings were taken in the youngest fully expanded leaf of each plant. SPAD values were converted in μmol chlorophyll m^{-2} using a calibration curve previously determined (Domingos, 2006). Fruits from each treatment were picked at the full red stage, weighted, analysed for TSS and immediately frozen at -80ºC for biochemical analysis.

Total organic acids and anthocyanins contents were determined in fruit juice. Malic and citric acids were extracted in accordance to Longo and Vasapollo (2006), and the ascorbic acid (AA) as described by Pestana et al. (2002), and analysed by HPLC with a System Gold Programmable Detector Module 166-UV-Vis (Beckman Coulter, USA). Different acids were identified and quantified comparing peaks produced by known pure standard solutions. In addition, fruits were analysed for total phenols compounds, determined according to the Folin-Ciocalteu method, using gallic acid as a standard, expressing the results as mg of gallic acid equivalent (GAE) per litre of juice. Juice was also analysed for antioxidant activity by using the free radical scavenging activity ($DPPH^{\bullet}$), the Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays. The free radical scavenging activity was evaluated by $DPPH^{\bullet}$ (2,2-diphenyl-1-picryl hydrazyl) method as described by Brand-Williams et al. (1995) and the antiradical activity was defined as the amount of antioxidant necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50% (IC50) after 45 minutes. The TEAC assay was determined according to the procedure illustrated by Re et al. (1999) which is based on the suppression of the absorbance of radical cations of 2,2´-azinobis(3-ethylbenzothiazoline 6-sulfonate) ($ABTS^{\bullet+}$) by antioxidants. The half maximal inhibitory concentration (IC50) of antioxidant able to decrease the initial $ABTS^{\bullet+}$ was determined after 5 minutes. A modified procedure of the ORAC assay described by Cao et al. (1996) was used by measuring the efficiency of antioxidant components in the juice to restrain the decline of the fluorescence induced by a peroxyl generator, 2,2´-Azobis(2-aminopropane) dihydrochloride (AAPH). Trolox equivalents were calculated using the relative area under the curve for samples compared to a Trolox standard curve, prepared under the same experimental conditions.

The means were compared by the t-Test at $P \leq 0.05$ and by using SPSS software version 16.0.

RESULTS AND DISCUSSION

Plants grown in the absence of Fe developed chlorotic symptoms in young leaves approximately after three weeks (Fig. 1). The control plants remained green during all the experimental period. At harvest, the average leaf chlorophyll concentration in green plants was 476 ± 9 μmol m^{-2}, but in chlorotic plants it was 205 ± 39 μmol m^{-2} corresponding to a 43% decrease.

The visual appearance of fruits and TSS of fruit juice were similar in both treatments; however, significant differences in the internal composition of fruits were

observed. The main organic acids in strawberry fruits are citric and malic acids (Montero et al., 1996). The malic acid to citric acid ratio, normally used as a fruit ripening indicator, varied between 0.2 for Fe sufficient plants and 0.3 for chlorotic plants and was inversely related with leaf chlorophyll concentrations in young leaves (data not shown). These results indicate a delay in ripening as observed by Álvarez-Fernández et al. (2003) in pear and peach with iron chlorosis.

Strawberry fruits are also an excellent source of ascorbic acid which is a health-promoting compound (Montero et al., 1996). In our study the ascorbic acid concentration was greatest in fruits collected in chlorotic plants. In Table 1 the anthocyanin profile of Selva strawberries, grown with and without Fe, is presented. The major anthocyanin in fruits was Pelargonidin (3-glucoside and 3-rutinoside), which represented about 80% of the total, in agreement with results obtained by Lopes da Silva et al. (2007) for other strawberry cultivars. In smaller proportions the presence of cyanidin (3-glucoside and 3,5-glucoside) was also detected. Iron deficiency did not significantly affect the relative proportions of each type of anthocyanin. However, chlorotic fruits had a smaller total anthocyanins content, comparatively to control fruits (Table 1), which was due to decreases in pelargonidin (around 60%) but not in cyanidin. These results can be explained by the decrease of the monoxygenase activity, responsible for cinnamic acid hydroxylation on 4-coumaric acid, one of the anthocyanin precursors, a process catalysed by the cytochrome P-450 with Fe involvement (Dewick, 2002). In addition, the total anthocyanins content of strawberry fruits increased with SPAD values in young leaves ($r=0.84$; $P=0.009$). No significant differences were observed in fruit phenolic content (Table 1) which varied from 1250 GAE L^{-1} juice to 1500 mg GAE L^{-1} juice.

Fruits from non-chlorotic plants had more capacity to scavenge peroxyl radicals than chlorotic plants. Wang and Lin (2000) have shown that strawberries also have antioxidant activity due to the relative high ORAC values. A positive correlation between this activity and total phenolic or anthocyanin content was detected as already reported by those authors. Fruits from chlorotic plants had more capacity to scavenge DPPH and ABTS radicals (expressed as smaller values) than green plants. The greater antioxidant activity measured by these two methods followed the increase observed in ascorbic acid concentrations. Similar results were observed by Ferreyra et al. (2007). These results may not be contradictory with those obtained by the ORAC method, as the mechanisms involved in these assays are different. Hydrogen atom transfer is the mechanism observed in the ORAC method, whereas in TEAC and DPPH single electron transfer reactions predominate. Those authors refer that weak correlations between ORAC and TEAC may be obtained, mainly when samples contain different types of antioxidants due to their different kinetics and reaction mechanisms.

CONCLUSIONS

In spite of a similar external appearance, fruits grown in the absence of Fe showed changes in internal quality parameters associated with a delay in fruit ripening, namely less total anthocyanins and total phenols. However, ascorbic acid increased as well as the antioxidant activity expressed by DPPH and TEAC methods. Our preliminary results have indicated that iron chlorosis affects some compounds related to flavour and health. Further studies should focus on iron management to increase strawberry quality even if this leads to a reduction in berry size and yield. Additionally, these results indicate that harvesting based on external colour and Brix degree may not be linked to a good quality of the fruit, especially under Fe depletion. This is particularly important due to the role of Fe in human health.

ACKNOWLEDGEMENTS

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Tables

Table 1. The effect of Fe treatment on some quality attributes of strawberry fruits.

	Chlorotic plants (Fe0)	Green plants (Fe2.5)	Significance
Total solids soluble (ºBrix)	10 ± 0.6	10 ± 0.1	ns
Malic/citric ratio	0.32 ± 0.02	0.21 ± 0.00	*
Ascorbic acid (mg 100 g^{-1} FW)	37 ± 0.1	29 ± 0.0	*
Total anthocyanins (µg g^{-1} FW)	431 ± 21	651 ± 66	*
Pelargonidin 3-glucoside	259 (60 %)	411 (63 %)	*
Pelargonidin 3-rutinoside	102 (24 %)	167 (26 %)	*
Cyanidin 3,5-diglucoside	57 (13 %)	55 (8 %)	ns
Cyanidin 3-glucoside	13 (3 %)	19 (3 %)	ns
Total phenols (mg GAE L^{-1} juice)	1251 ± 260	1514 ± 71	ns
Antioxidant activity			
$DPPH^{\bullet}$ (IC50)	260 ± 13	359 ± 10	*
ORAC (µM Trolox ml^{-1} juice)	27 ± 3	48 ± 0	*
TEAC (IC50)	272 ± 7	341 ± 12	*

FW – fresh weight; GAE – gallic acid equivalents; $DPPH^{\bullet}$ - Free radical scavenging activity; TEAC - Trolox equivalent antioxidant capacity; ORAC - oxygen radical absorbance capacity; IC50 - half maximal inhibitory concentration; ns – not significant; * - significantly different at $P<0.05$ (t-Test).

Figures

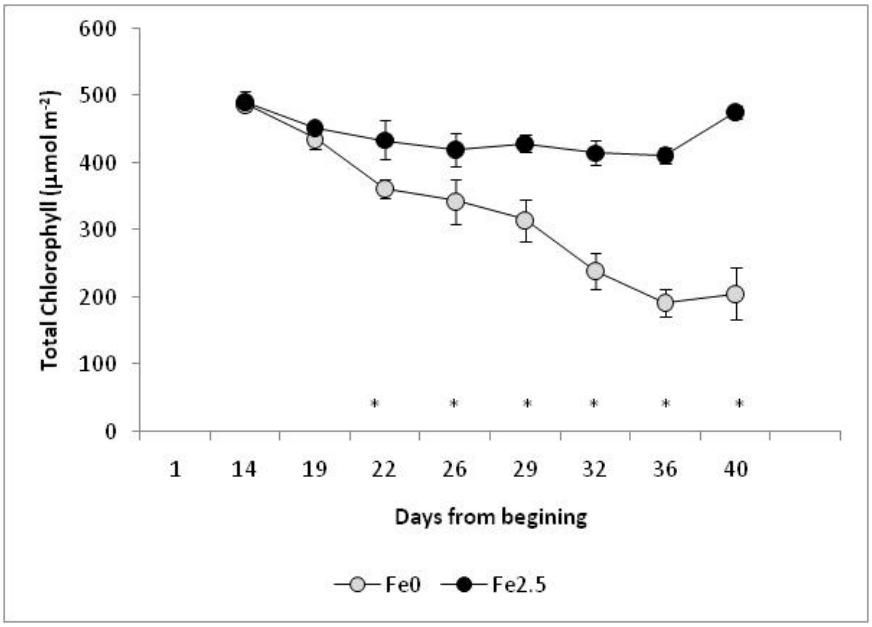

Fig. 1. Total leaf chlorophyll variation during the experiment. *: Significantly different at $P<0.05$ (t-Test).

A caraterização e correção da deficiência de ferro em plantas de morangueiro: novas abordagens

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Resumo

O ferro (Fe) é um elemento abundante nos solos e apesar de ser necessário em pequenas quantidades para as plantas, a incidência de clorose férrica (deficiência de Fe) é comum em muitas espécies agrícolas sendo necessário recorrer à aplicação massiva ao solo de quelatos de Fe sintéticos.

Neste trabalho apresentam-se de forma resumida os resultados obtidos em diversos ensaios com plantas de morangueiro (*Fragaria* × *ananassa* Duch.) cujos objetivos foram: o estudo dos mecanismos fisiológicos e bioquímicos de controlo da deficiência de ferro e a avaliação de novas alternativas para a correção da clorose férrica.

Em todos os ensaios, conduzidos em sistema hidropónico, os sintomas foram induzidos pela ausência do Fe na solução e os resultados comparados com um tratamento controlo com Fe. O grau de clorose e a recuperação dos sintomas foram estimados através dos valores de SPAD. A atividade da quelato de Fe(III)-redutase (QF-R), enzima responsável pela redução do Fe nas raízes, foi determinada nos ápices radiculares pela quantificação colorimétrica do complexo Fe(II)-BPDS. O teor de Fe foi determinado por espectrofotometria de absorção atómica, após calcinação das amostras a 450 ºC e digestão ácida das cinzas.

As plantas de morangueiro que cresceram sempre sem Fe apresentaram sintomas de clorose férrica e alterações da morfologia externa das raízes, acompanhadas por incrementos na atividade radicular da QF-R. A recuperação de plantas cloróticas foi efetuada através da aplicação do mesmo produto (sulfato ferroso) em dois locais distintos, foliarmente e à solução. Nas plantas recuperadas pela aplicação de Fe à solução, a atividade da QF-R manteve-se alta, sugerindo uma estratégia destinada a incrementar as reservas deste elemento. Em alternativa aos quelatos férricos sintéticos foi testada a aplicação foliar de um extrato vegetal preparado a partir de aparas de relva (patente PT/103584-2009 da UALG e patente internacional PCT/PT2007/000041-2008; em compropriedade entre a UALG e a empresa ADP-Fertilizantes) que foi eficaz no reverdescimento após três aplicações. Neste contexto, os resultados são discutidos de forma a salientar as implicações práticas destas respostas fisiológicas e bioquímicas, numa perspetiva global da fertilização do Fe.

Palavras-chave: clorose férrica, SPAD, quelato de ferro redutase.

Abstract

Characterization and correction of Fe deficiency in strawberry: novel approaches.

Iron (Fe) is abundant in soils and although it is required in small amounts by plants the incidence of iron chlorosis (Fe deficiency) is very common in a number of crops and requires massive soil application of Fe-chelates to correct it. In this work, we present the most important results obtained in several experiments conducted with strawberry to study the physiological and biochemical response mechanisms to Fe deficiency, and the assessment of novel alternatives to control this nutritional disorder.

In all experiments, conducted in hydroponic systems, symptoms were induced by withdrawing Fe from the solution and the results were compared to a control treatment grown with Fe. The degree of chlorosis and symptoms recovery was estimated using SPAD values. The activity of iron chelate reductase, the enzyme responsible for Fe reduction in roots, was determined in root apices by colorimetric quantification of the BPDS complex. The Fe concentration in leaves and roots was quantified by atomic absorption spectrophotometry after treatments at 450 ºC and acid digestion of the ashes obtained.

Strawberry plants that grew always without Fe, presented Fe chlorosis and morphological external root modifications associated with increases of the activity of the Fe-reductase enzyme. The recovery of chlorotic plants was achieved by application of Fe sulphate either to leaves or to the nutrient solution. In plants recovered by using Fe in the solution, the enzyme maintained a large activity, suggesting a strategy to increase plant Fe pools.

As an alternative to synthetic Fe chelates, we also tested a foliar application of a plant extract obtained from fresh grass clippings (national patent PT/103584-2009 of UALG, and international patent PCT/PT2007/000041-2008, UALG and ADP-Fertilizantes), which was effective in chlorosis recovery after three applications. The results are discussed in order to highlight the practical implications of these responses under a perspective of optimization of crop Fe fertilization.

Keywords: iron chlorosis, SPAD, ferric chelate redutase.

Introdução

O processo de absorção do Fe nas dicotiledóneas (Abadía et al., 2011; Pestana et al., 2004), inicia-se pela sua redução na membrana plasmática, através da ação de um quelato de Fe(III)-redutase. Uma vez no simplasto do sistema radicular, o Fe(II) é oxidado e complexado pelo ácido cítrico a Fe(III)-citrato, forma em que é translocado, *via* xilema, para a parte aérea. O Fe^{2+} que entra no citoplasma é complexado pela nicotianamina e nesta forma é uniformemente distribuído no simplasma, permitindo a sua participação nos diversos processos metabólicos.

Numa situação de deficiência de Fe, as plantas desenvolvem sintomas de clorose férrica, que devido à baixa mobilidade do Fe na planta, surgem nas folhas mais jovens, caracterizando-se pelo aparecimento de um fino reticulado no qual apenas as nervuras permanecem verdes (Abadía, 1992). As diferentes espécies vegetais e por vezes algumas cultivares apresentam comportamentos distintos face à clorose férrica o que permite classificá-las, em espécies eficientes, pela sua capacidade de adaptação à deficiência de Fe e espécies não eficientes, que por terem mecanismos de resposta efetivos, morfológicos ou fisiológicos, desenvolvem os sintomas característicos de clorose férrica. Adicionalmente, esta deficiência nutritiva afeta vários processos

metabólicos e origina decréscimos na produção e na qualidade dos frutos (Alvarez-Fernández et al., 2006; Pestana et al., 2003; 2010).

Atualmente, a correção da clorose férrica em fruteiras faz-se sobretudo recorrendo a aplicações massivas ao solo de quelatos férricos sintéticos, como o ácido etileno-diamina di-orto-hidroxi-fenil de ferro (Fe-EDDHA). Devido à rápida imobilização do Fe em solos calcários, estas aplicações têm de ser repetidas nas mesmas árvores, quase sempre anualmente, estimando-se que os custos da correção da clorose férrica representem 60% dos custos totais da fertilização (Tagliavini et al., 2000). Na Região de Zaragoza, estima-se que sejam gastos cerca de 14 M€ para corrigir a clorose férrica nos 90.000 ha de fruteiras instaladas em solos calcários (Abadía et al., 2004).

O impacto ambiental destas aplicações pode ser elevado, já que os agentes quelatantes sintéticos são bastante estáveis e podem poluir os solos, rios e lençóis freáticos (Lucena, 2006). Por esta razão, realizaram-se diversos estudos que procuram tratamentos alternativos, sem recurso a quelatos sintéticos. Na UALG, recorreu-se ao resíduo proveniente da manutenção de espaços verdes, aparas de relva, e preparou-se um extrato vegetal, que aplicado foliarmente, foi eficaz no reverdescimento de plantas de morangueiro com clorose férrica (patente PT/103584-2009 da UALG e patente internacional PCT/PT2007/000041-2008; em compropriedade entre a UALG e a empresa ADP-Fertilizantes).

Neste trabalho apresentam-se de forma resumida os resultados obtidos em diversos ensaios com plantas de morangueiro (*Fragaria* × *ananassa* Duch.) cujos objetivos foram: o estudo dos mecanismos fisiológicos e bioquímicos de controlo da deficiência de Fe e a avaliação de novas alternativas para a correção da clorose férrica.

Material e Métodos

Para atingir os objetivos propostos usaram-se plantas de morangueiro (*Fragaria* x *ananassa* Duch) da cultivar 'Selva' adquiridas em viveiro de raiz nua e transplantadas para caixas de 20 litros com solução de Hoagland, correspondendo às seguintes concentrações (em mM): 2,5 $Ca(NO_3)_2.4H_2O$, 2,5 KNO_3, 0,5 KH_2PO_4, 1,0 $MgSO_4.7H_2O$ e, (em µM) 23 H_3BO_3, 0,4 $ZnSO_4.7H_2O$, 0,2 $CuSO_4.5H_2O$, 4,5 $MnCl_2.4H_2O$ e 1 MoO_3. Estabeleceram-se duas modalidades com base na adição de 10 µM Fe (Fe10), na forma de quelato Fe-EDDHMA ou pela ausência do Fe (Fe0), induzindo o aparecimento dos sintomas. O arejamento das soluções foi assegurado por uma conduta de tubagens ligadas a um compressor. O pH e a condutividade elétrica (CE) foram usados para monitorizar as soluções nutritivas que foram substituídas sempre que os valores iniciais de CE decresceram 0,2 dS m^{-1}.

O estudo da recuperação de morangueiros cloróticos foi efetuado pelo estabelecimento de dois ensaios. Num dos ensaios (ensaio 1), a recuperação foi avaliada através da adição de sulfato ferroso de dois modos: à solução nutritiva (+Fe-solução) ou por pulverização foliar (+Fe-folhas). A aplicação do Fe à solução nutritiva foi efetuada com uma solução de sulfato ferroso (0,75 mM de Fe) enquanto a pulverização foliar foi realizada três vezes com uma solução de sulfato ferroso (1,8 mM) conforme descrito em Pestana et al. (2012). No outro ensaio (ensaio 2), as plantas cloróticas foram pulverizadas com um extrato vegetal (+Fe-extrato) preparado a partir de aparas de relva (Pestana et al., 2008; 2009; 2011).

O grau de clorose e de recuperação dos sintomas foram estimados através dos valores de SPAD medidos nas folhas jovens e convertidos em clorofila total (CHL) através da curva de calibração $CHL = 0,45 \times SPAD^2 - 1,11 \times SPAD + 32,56$ (Pestana et al., 2011).

A atividade da quelato de Fe(III)-redutase (QF-R), enzima responsável pela redução do Fe nas raízes, foi determinada nos ápices radiculares pela quantificação colorimétrica do complexo Fe(II)-BPDS (Pestana et al. 2012).

A partição da biomassa foi avaliada através da razão entre os pesos secos da raiz e da parte aérea, determinados após 48 h a 70 ºC, no final dos ensaios. De seguida, o material vegetal foi moído e calcinado, tendo o teor de Fe sido determinado por espectrofotometria de absorção atómica, após calcinação das amostras a 450 ºC e digestão ácida das cinzas (Pestana et al., 2001).

As plantas foram sempre aleatoriamente distribuídas pelas modalidades. A comparação das médias obtidas para determinado parâmetro foi efetuada através da análise de variância (ANOVA) para um nível de significância de 95%. A análise estatística foi realizada recorrendo ao programa SPSS 17.0.

Resultados e Discussão

As plantas de morangueiro que cresceram sempre sem Fe apresentaram sintomas de clorose férrica e decréscimos elevados no teor de clorofila (quadro 1), tal como seria de esperar (Abadía & Abadía, 1992). Simultaneamente foram registadas alterações na morfologia externa das raízes, acompanhadas por incrementos na atividade radicular da QF-R (quadro 2).

A recuperação das plantas cloróticas foi efetuada através da aplicação de sulfato de ferro(II) em dois locais distintos, foliarmente e à solução, e o reverdecimento foi observado e traduzido por incrementos nos teores de clorofila total das folhas jovens (quadro 1). Por sua vez, observou-se que a atividade radicular da QF-R respondeu mais rapidamente ao Fe aplicado foliarmente do que ao Fe adicionado às raízes (quadro 2). Nas plantas recuperadas pela aplicação de Fe à solução, a atividade da QF-R manteve-se alta, sugerindo uma possível estratégia destinada a incrementar as reservas deste elemento na planta (Pestana et al., 2012). Os teores de Fe nas folhas aumentaram com a aplicação de Fe foliarmente, o mesmo se observando quando este foi aplicado à solução nutritiva apesar da concentração atingida ser mais baixa (quadro 2). No entanto, a razão da biomassa parte aérea/raiz com aplicação de Fe à solução tornou-se idêntica à da obtida nas plantas que foram sempre cultivadas com Fe (quadro 2).

Como alternativa aos quelatos férricos sintéticos, a aplicação foliar do extrato vegetal foi eficaz após três aplicações, tendo-se observado valores de clorofila equivalentes aos das plantas que cresceram sempre com Fe na solução (quadro 1) embora o teor de Fe na planta não tenha aumentado sugerindo um uso mais eficiente do Fe já presente na planta (quadro 2). Parece haver um certo desfasamento entre as alterações morfológicas e as variações na atividade da QF-R induzidas pela deficiência, pois após o reverdecimento das folhas motivado pela aplicação foliar do extrato vegetal, as alterações morfológicas observadas a nível radicular (típicas da estratégia I) foram desativadas mas o mesmo não aconteceu com a atividade da QF-R, que permaneceu alta. Assim, as alterações morfológicas da raiz parecem ser só reguladas pelo teor foliar de Fe, enquanto as respostas fisiológicas como a atividade da QF-R necessita de sinais vindos da raiz e da parte aérea em simultâneo (Pestana et al., 2011).

Neste contexto, numa perspetiva global da fertilização do ferro, é importante avaliar as diferenças entre as respostas fisiológicas e bioquímicas à aplicação foliar e/ou ao solo de forma a otimizar a produção.

Conclusões

A aplicação foliar de um extrato vegetal preparado a partir de aparas de relva pode ser uma alternativa aos quelatos férricos sintéticos na correção da clorose férrica em morangueiros. Através dos resultados obtidos constatou-se que é possível otimizar a época de aplicação do Fe, minimizando as perdas e desenvolver novos métodos que permitam dinamizar as reservas nativas de Fe na planta.

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Quadro 1- Valores médios de clorofila das folhas jovens registados ao longo do tempo em dois ensaios (Pestana et al., 2011; 2012) de recuperação da clorose férrica em morangueiros cv 'Selva'. A % de variação foi calculada em relação ao controlo (plantas verdes). Para cada ensaio e cada parâmetro, médias seguidas pela mesma letra não são significativamente diferentes a 95% (teste de Duncan).

Número de dias	(+15) Primeiras folhas	(+36) Plantas com clorose Fe	(+55) Inicio da recuperação	(+71) Final do ensaio	% de variação
Ensaio 1					
Fe0	414 a	139 b	118 b	94 c	
Fe10	423 a	318 a	408 a	397 a	
+Fe-folhas				204 b	*+ 54 %*
+Fe-solução				331 a	*+ 82 %*
Ensaio 2					
Fe0	344 b	148 b	64 b	32 b	
Fe10	545 a	600 a	527 a	470 a	
+Fe-extrato		98 b	81 b	449 a	*+ 95%*

Quadro 2- Valores médios de atividade da QF-R, de concentração de Fe nas folhas e na raiz e da razão raiz/parte aérea, em peso seco, registados no final dos dois ensaios (Pestana et al., 2011; 2012) de recuperação da clorose férrica em morangueiros 'Selva'. Para cada ensaio e cada parâmetro, médias seguidas pela mesma letra não são significativamente diferentes a 95% (teste de Duncan).

	QF-R	Fe (mg kg^{-1} ps)		Raiz/parte aérea
	(nmol Fe(II) g^{-1} pf min^{-1})	Folhas	Raízes	(peso seco)
Ensaio 1				
Fe0	52 a	59 d	374 c	0.9 a
Fe10	10 c	84 c	593 b	0.4 b
+Fe-folhas	5 d	275 a	395 c	0.7 a
+Fe-solução	25 b	173 b	1658 a	0.5 b
Ensaio 2				
Fe0	28.3 a	37 a	1205 a	1.2 a
Fe10	24.9 a	51 a	1003 a	0.7 b
+Fe-extrato	18.6 a	72 a	683 a	0.8 a

pf – peso fresco; ps- peso seco

Deficiência de Fe em plantas de morangueiro: efeitos na partição da biomassa e na produção.

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Palavras-chave: biomassa, clorose férrica, *Fragaria* x *ananassa*.

Resumo

O termo clorose férrica é aplicado a plantas nas quais os processos fisiológicos em que participa o ferro se encontram reduzidos ou inactivos. É uma das mais importantes deficiências nutritivas pois é susceptível de limitar a produção agrícola e a qualidade do fruto. Neste trabalho pretendeu-se estudar a deficiência de Fe de plantas de morangueiro (*Fragaria* x *ananassa* Duch) em solução nutritiva, avaliando a biomassa, a produção total e a qualidade dos frutos ao longo do ensaio. Colocaram-se plantas de morangueiro, cultivar 'Selva', em solução nutritiva de Hoagland, com e sem ferro. As plantas com deficiência de Fe tiveram o padrão de acumulação da biomassa alterado, anteciparam a produção e produziram mais frutos mas de calibre inferior.

INTRODUÇÃO

A clorose férrica é uma deficiência nutricional que pode resultar de uma baixa disponibilidade de Fe ou de mecanismos que conduzem à imobilização fisiológica do Fe (Pestana et al., 2003, 2004), o que resulta num decréscimo dos pigmentos fotossintéticos e no aparecimento dos sintomas característicos de clorose férrica (Abadía, 1992). Devido à pouca mobilidade do Fe na planta, os sintomas desta deficiência manifestam-se inicialmente nas folhas mais jovens e caracterizam-se pelo aparecimento de um fino reticulado no qual apenas as nervuras permanecem verdes. A clorose do limbo parece ser devida, sobretudo, ao facto do Fe ser essencial para a formação das membranas dos tilacóides (Abadía, 1992). Esta deficiência nutritiva afecta vários processos metabólicos origina decréscimos na produção e na qualidade do fruto (Alvarez-Fernández et al., 2006), não estando ainda reportados os efeitos na cultura do morangueiro.

Neste trabalho pretendeu-se estudar a deficiência de Fe em plantas de morangueiro (*Fragaria* x *ananassa* Duch) em solução nutritiva, avaliando o teor de clorofila total e o número de estolhos emitidos ao longo do ensaio, assim como o padrão de partição da biomassa e a produção total no final do ensaio.

MATERIAL E MÉTODOS

Colocaram-se plantas de morangueiro (*Fragaria* x *ananassa* Duch) cultivar 'Selva', adquiridas em viveiro de raiz nua, em solução nutritiva de Hoagland com as seguintes concentrações (em mM): 2.5 Ca (NO_3) $2.4H_2O$, 2.5 KNO_3, 0.5 KH_2PO_4, 1.0 $MgSO_4.7H_2O$, e (em μM) 23.0 H_3BO_3, 0.4 $ZnSO_4.7H_2O$, 0.2 $CuSO_4.5H_2O$, 4.5 $MnCl_2.4H_2O$ e 1.0 MoO_3. Estabeleceram-se 2 modalidades com base nas diferentes concentrações de Fe (0 e 2,5μM de Fe), adicionado na forma de quelato Fe-EDDHMA. No inicio do ensaio, o pH das soluções foi ajustado para 6.0. O ensaio decorreu durante 7

semanas numa estufa de vidro, tendo sido colocadas 6 plantas por caixa de 20 litros de solução nutritiva, num total de 18 plantas por modalidade.

Os valores de SPAD foram medidos pelo menos 2 vezes por semana nas folhas jovens e convertidos em clorofila total (CHL) através da curva de calibração CHL = 0,45 x $SPAD^2$ – 1,11 x SPAD + 32,56 (Domingos, 2006).

A partição da biomassa foi avaliada através dos pesos secos registados nas raízes, coroas, folhas e flores. Foi calculada a distribuição relativa (%) de cada uma das partes consideradas no peso seco total de cada planta e para cada uma das modalidades. Para cada uma das modalidades, os frutos foram colhidos em pleno estado de maturação, contados e pesados.

Análise estatística

As plantas foram aleatoriamente distribuídas pelas modalidades. A comparação das médias obtidas para determinado parâmetro foi efectuada através da análise de variância (ANOVA) para um nível de significância de 95%. A análise estatística foi realizada recorrendo ao programa SPSS 15.0.

RESULTADOS E DISCUSSÃO

Os sintomas de clorose férrica foram observados 35 dias após o início do ensaio, apenas nas plantas que cresceram sem Fe. Os teores de clorofila total registados durante todo o ensaio estão apresentados na Figura 1.

Os primeiros estolhos apareceram nas plantas verdes (Fe2,5) 33 dias após o início do ensaio sendo emitidos mais tarde nas plantas cloróticas e sempre em número muito inferior (Fig. 2).

As plantas cloróticas produziram mais frutos maduros e com mais peso do que as plantas não cloróticas; indicando uma antecipação na maturação dos frutos o que foi comprovado pelos valores mais altos da razão açucares/ácidos totais, respectivamente de 0,89 e 0,73.

Como as plantas foram colocadas a crescer em solução nutritiva sem Fe, é possível dizer que as reservas de Fe e de fotoassimilados tenham sido distribuídas preferencialmente para o desenvolvimento reprodutivo. Este facto não se verificou nas plantas não cloróticas, tendo-se observado mais crescimento vegetativo e um atraso na maturação do fruto. Estes resultados são diferentes dos observados em fruteiras como citrinos e pessegueiros (Álvarez-Fernández et al., 2006), o que poderá estar relacionado com o ciclo cultural do morangueiro.

Em relação à partição de biomassa (Fig. 4) é possível verificar que nas plantas cloróticas, as folhas e as flores apresentaram menor peso seco do que nas não-cloróticas e que a biomassa foi preferencialmente mobilizada para a coroa e raiz. Diversos trabalhos associam a diminuição da área foliar a uma absorção deficiente em Fe (Pestana et al., 2003).

A clorose férrica antecipou a entrada em floração e em produção tendo originado mais frutos por planta e mais pequenos (diâmetro inferior a 18 mm - categoria III; dados não apresentados). Por outro lado, as plantas verdes emitiram mais estolhos mas produziram menos frutos. Estes resultados poderão ter a ver com a partição de biomassa e o investimento preferencial das plantas cloróticas no crescimento reprodutivo em detrimento do vegetativo.

Agradecimentos

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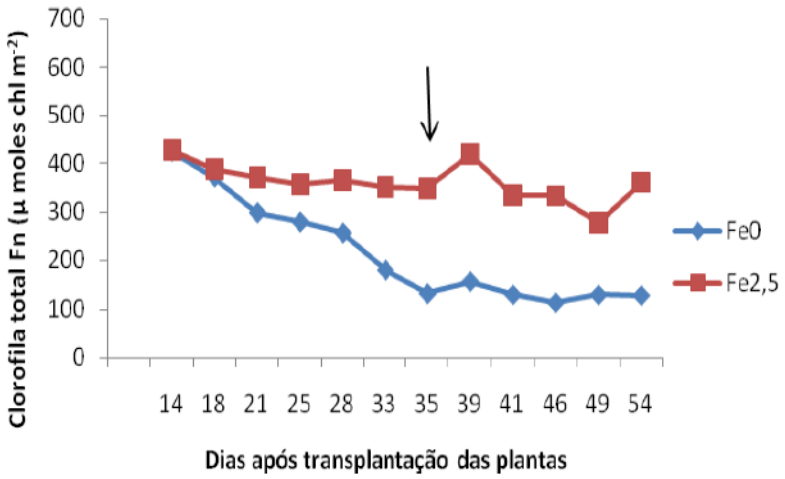

Fig. 1. Variação da clorofila total (µmol m^{-2}) nas folhas novas – Fn, nos morangueiros durante todo o ensaio nas diferentes modalidades (Fe0- 0µM de Fe e Fe2,5- 2,5µM de Fe). A seta indica a data de aparecimento dos sintomas de clorose férrica, observados apenas nas plantas que cresceram sem Fe (Fe0). A partir dos 28 dias as médias entre modalidades foram significativamente diferentes (P <0,05).

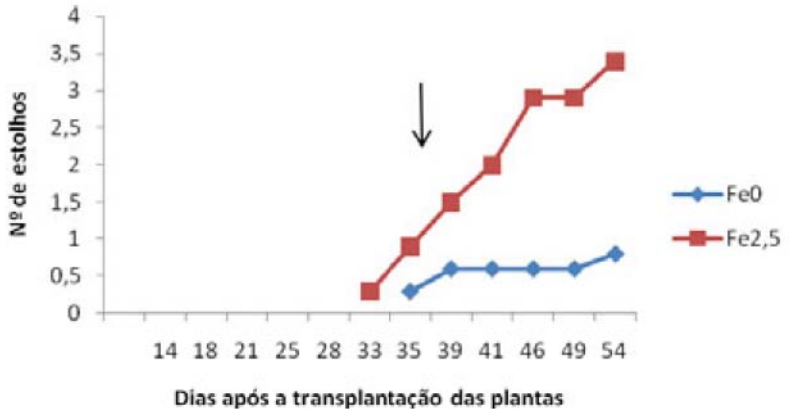

Fig. 2. Número médio acumulado de estolhos emitidos durante todo o ensaio nas modalidades em estudo (Fe0- 0µM de Fe e Fe2,5- 2,5µM de Fe). A seta indica a data de aparecimento dos sintomas de clorose férrica nas plantas que cresceram sem Fe (Fe0). A partir dos 35 dias as médias entre modalidades foram significativamente diferentes (P <0,05).

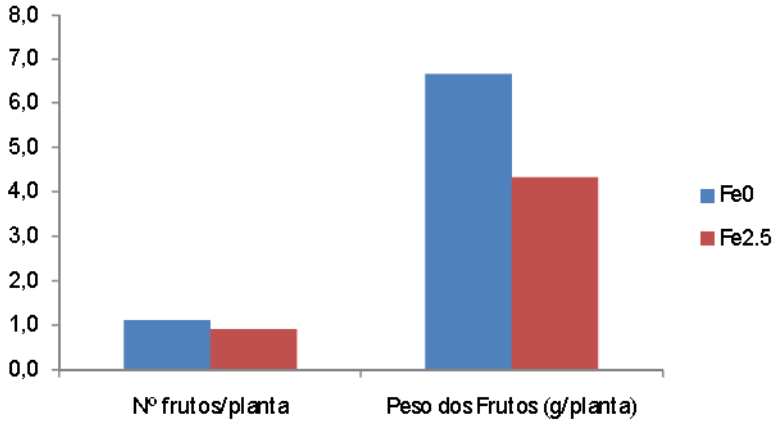

Fig. 3. Número e peso médio de frutos maduros colhidos no final do ensaio das duas modalidades (Fe0- 0µM de Fe e Fe2,5 e 2,5µM de Fe). O peso dos frutos foi significativamente diferente nas duas modalidades (P <0,05).

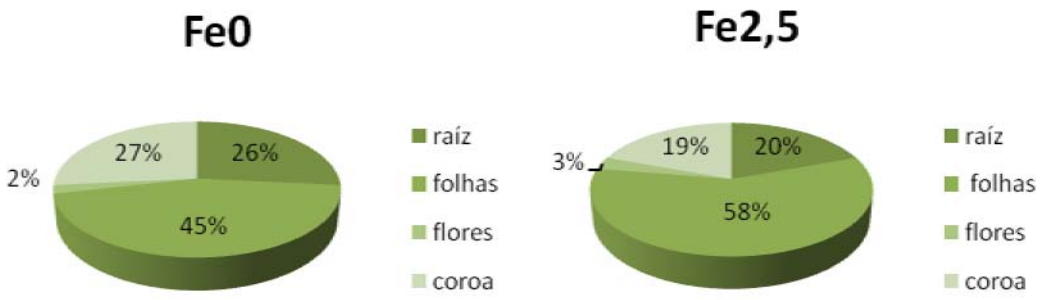

Fig. 4. Distribuição relativa (%) do peso seco das raízes, folhas, flores e coroa das plantas de morangueiro sujeitas a diferentes modalidades (Fe0- 0µM de Fe; Fe2,5 e 2,5µM de Fe) no final do ensaio.

The Effects of Fe Deficiency on Organic Acids, Sugars and Anthocyanins in Strawberry Fruits

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ABSTRACT

The quality of strawberry (*Fragaria x ananassa* Duch.) fruits can be defined by its texture, taste (soluble sugars and organic acids) and colour (anthocyanin content). Iron (Fe) deficiency is among the most frequent nutritional disturbance, in the Mediterranean region. Bare root transplants (without leaves) with approximately 18 cm, were transferred to Hoagland's nutrient solution, using Fe-EDDHMA as the Fe source, at three different concentrations: 0, 2.5 and 5 µM Fe. Plants were grown in 20 L containers in a glasshouse for 6 weeks (from April 27 to June 5) under natural photoperiod conditions and air temperature ≤ 25 ºC. Eighteen transplants were used per treatment distributed in a complete randomized design. Leaf chlorosis was evaluated by using a Minolta SPAD-502 chlorophyll meter. At the end of the experiment, total organic acids, total sugars and total anthocyanins were assessed in fruits. Plants grown in absence of Fe revealed chlorotic symptoms approximately after three weeks of exposure. The total sugar and organic acid content in fruits was not affected by Fe deficiency, whereas the total anthocyanin content noticeably decreased. The malate/citrate ratio increased with Fe deficiency possibly indicating a delay in fruit ripening.

Keywords: anthocyanins, Fe deficiency, organic acids, strawberry, sugars.

INTRODUCTION

Iron deficiency results in a decrease in the concentration of photosynthetic pigments in leaves, usually referred to as iron chlorosis. The symptoms occur primarily in young leaves

and became apparent as an interveinal chlorosis with the appearance of a fine reticulation (Abadía and Abadía, 1993).

Iron chlorosis affects several metabolic processes and leads to nutrient imbalances in the plant. Decreased yield and poor quality of fruits resulting from the deficiency were reported for several crops. For example, in *Citrus* spp., El-Kassa (1984) reported the negative effect of iron chlorosis on gross yield and fruit quality of lime. Iron chlorosis can also lead to a delay in fruit ripening in orange and peach (Sanz et al., 1997; Pestana et al., 2001). In peach fruits, yet external aspect was similar, changes on chemical composition were reported, affecting organoleptic and nutritional properties (Álvarez-Fernández et al., 2006).

Strawberries (*Fragaria ananassa* Duch.) quality can be defined by its texture, taste (soluble sugars and organic acids) and colour (anthocyanin content) at harvest date (Kafkas et al., 2007).

Although the main constituents of strawberries during maturation are well known, fewer studies have been done on their variation induced by nutritional disorders. The objective of the present study was to evaluate the effect of Fe deficiency on some physical and chemical characteristics of strawberry fruits harvested at same maturity stage from plants grown with different Fe concentrations in nutrient solution.

MATERIALS AND METHODS

Plant material and growth conditions

The experiment was conducted in a glasshouse at the University of Algarve, in Faro (37º 02` N; 7º 58` W), south of Portugal. Strawberry bare root transplants (without leaves) of cv. 'Selva', a day-neutral cultivar, with approximately 18 cm root length, were disinfected by immersion in an antifungal solution containing fosetyl aluminium (2 g L^{-1}) during approximately 2 hours and rinsed with running water. The transplants were then transferred to Hoagland's (1/2 strength) nutrient solution with the following composition: (in mM): 2.5 Ca $(NO_3)_2.4H_2O$, 2.5 KNO_3, 0.5 KH_2PO_4, 1.0 $MgSO_4.7H_2O$, (and in µM) 23.0 H_3BO_3, 0.4 $ZnSO_4.7H_2O$, 0.2 $CuSO_4.5H_2O$, 4.5 $MnCl_2.4H_2O$ and 1.0 MoO_3. Fe was added to the solution by Fe-EDDHMA, at three different concentrations: 0, 2.5 and 5 µM Fe. Plants were grown in 20 L containers, each holding 6 plants, for 6 weeks, from April 27 to June 5 under natural

photoperiod conditions and air temperature ≤ 25ºC. The nutrient solution was aerated and the pH adjusted to 6.0. During this experiment nutrient solutions were controlled by pH and electrical conductivity readings twice a week.

Leaf chlorosis was evaluated by using a Minolta SPAD-502 chlorophyll meter. Six readings were taken in the youngest fully expanded leaf of each plant.

At the end of the experiment, plants were separated into shoots (including leaves) and roots. Plant material was rinsed with tap water, distilled water containing a non-ionic detergent and finally three times in distilled water. The dry weight of each part was determined after drying at 70 ºC. The ratio root dry weight/shoot dry weight was calculated for each plant and treatment to study the effect of Fe chlorosis on biomass allocation.

Chemical analysis

Fruits were harvested from each treatment 11 days after fruit set, weighted and used for chemical analysis. Total organic acids, sugars and anthocyanins contents were determined in fruit juice.

Organic acids extraction was evaluated in accordance to Longo and Vasapollo (2006), which consists of using 2 g of macerated fruit, extracted with 0.1% HCl (v / v) in methanol. The acids composition was detected by HPLC at 210 nm. The column used was a RP-18 (250 x 4 mm; 5 µm particle size). The mobile phase was 20 mM NaH_2PO_4 with a pH=2.7, at a flow rate of 0.5mL min^{-1}.

For sugars and anthocyanins quantification, 1 mL samples were centrifuged for 30 minutes at 10 000 rpm and filtered trough a 0.22 µm filter (Millipore) then stored at -20 ºC until analysis. Sugar composition was detected by HPLC equipped with a refractive index (RI) detector. The column used was a Lichrospher 100 NH_2 (250 x 4mm; 5µm particle size). The mobile phase was acetonitrile 83% at a flow rate of 1mL min^{-1}. Anthocyanins were identified by HPLC UV-Vis, with a RP-18 (250x4mm; 5µm particle size). The mobile phase was 5 % formic acid (A) and methanol (B), in a linear gradient from 15 % to 35 % B at 20 minutes, followed by isocratic run until 30 minutes. The flow rate was 1 mL min^{-1} and recorded at absorbance of 520 nm.

Different acids, sugars and anthocyanins were identified and quantified comparing peaks produced by known pure standard solutions. The sum of these components was

considered in this study. Considering organic acids, the malate to citrate ratio was used in order to evaluate the maturity stage of harvested fruits.

Statistical analysis

The means were compared by analysis of variance and by using the Duncan Multiple Range Test at $P \leq 0.05$. Regression analysis was carried out between several parameters. All the statistical analyses were done by using SPSS software version 16.0.

RESULTS AND DISCUSSION

Fe deficiency symptoms appeared three weeks after transplant only in plants grown without Fe (Fe0). At the end of the experiment, these plants had the lowest SPAD values (Table 1) with moderate symptoms of iron chlorosis. On the other hand, plants from the other treatments remained without symptoms (green) during the entire assay.

Fruit size is reduced by Fe deficiency in several crops such as: *Citrus* spp. (Pestana et al., 2002), kiwifruit (Tagliavini et al., 2000), peach (Álvarez-Fernández et al., 2005) and pear (Álvarez-Fernández et al., 2003). However, in our study the absence of Fe did not affect the fruit fresh weight which was similar for all treatments (6.5 g ± 1.3). This result was probably due to a high endogenous pool of Fe previously accumulated, during nursery growth as previously observed by Domingos (2006).

The variation of total organic acids and total sugars was not significantly different, however the values were lower in chlorotic fruits (-Fe) compared with other treatments (+Fe). The accumulation of organic acids was reported in leaves and roots of Fe deficient plants (Abadía et. al., 2002).

Chlorotic fruits presented a lighter red skin colour, revealing a delay in ripening (red colour development), compared to fruits of plants grown with Fe in nutrient solution. This visual appearance is in accordance with the decrease observed in total anthocyanins (Table 1), and could be explained by the decrease of the monoxygenase activity, responsible for cinnamic acid hydroxylation on 4-coumaric acid, one of the anthocyanin precursors, a process catalysed by the cytochrome P-450 with Fe involvement (Dewick, 2002). In addition,

the total anthocyanins content of strawberry fruits increased with SPAD values in young leaves, a measurement of Fe chlorosis (r = 0.84; *P* =0.009; n=9).

Table 1

Mean of SPAD values of young leaves (n=18) and total contents of sugars, organic acids and anthocyanins of juice fruits (n=3) harvested from strawberry plants grown with different Fe treatments: Fe0 – 0 µM of Fe , Fe2.5 – 2.5 µM of Fe and Fe5 – 5 µM of Fe. For each treatment, means with the same letter are not significantly different at $P \geq 0.05$ (Duncan test). FW – fresh weight.

	Fe0	**Fe2.5**	**Fe5**
SPAD values of young leaves	16 b	33 a	28 a
Total content of fruits (mg g^{-1} fruit FW)			
sugars	10.8 ab	14.1 a	9.3 b
organic acids	12.5 a	19.5 a	15.1 a
anthocyanins	0.43 b	0.65 ab	0.68 a

The absence of Fe in the nutrient solution may induce the root response mechanisms to improve Fe uptake (morphological changes were visible only in chlorotic plants). As a result the root/shoot ratio (w / w) markedly increased in chlorotic plants (Figure 1).

Malate/citrate ratio can be used to identify the maturity stage. The markedly increase in the malate/citrate ratio (Figure 1) was also reported by Álvarez-Fernández et al. (2005) in pear fruits from chlorotic trees. Since the fruits were harvested at the same stage (11 days after fruit set), we may assume that the delay in fruit ripening was due to Fe deficiency. Moreover a correlation between malate/citrate ratio and root/shoot ratio was obtained (r = 0.88; *P* =0.013; n=9), indicating that this delay was probably related to the different biomass allocation in chlorotic plants.

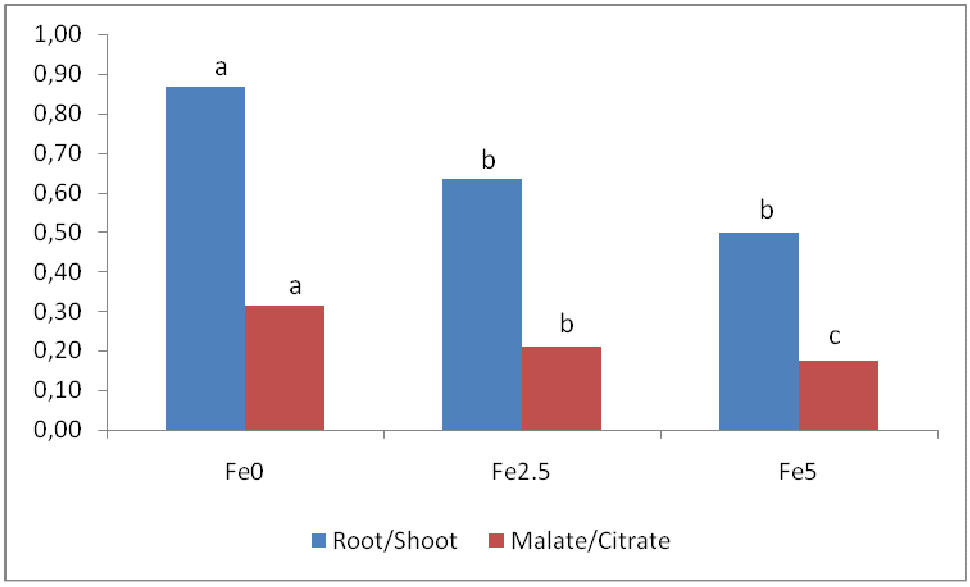

Figure 1. Effects of Fe level on the root to shoot ratio (dry weight, w / w) and on malate to citrate acids ratio (w / w) in fruits at the end of the experiment. For each treatment, means with the same letter are not significantly different at $P \geq 0.05$ (Duncan test).

Strawberry plants with symptoms of iron chlorosis produce fruits with similar weight but with less intense colour and poor organoleptic characteristics. Strawberries are classified as non-climateric fruits (Cordenunsi et al., 2002), so the nutritional imbalance induced by Fe deficiency may affect not only the harvest date but also fruit storage and commercialization.

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